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# Risk factors for multiple sclerosis in the Northern Isles of Scotland

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## Declaration

I declare that:

1) I alone composed this thesis;

2) The work herein is my own. For those elements which have resulted from a collaboration, I declare that I have made a substantial contribution, and have clearly indicated in the text my own contribution and that of others. Figures which are not my original work have been clearly labelled in the text, and have been reproduced with permission from the publishers or under the 'Criticism and Review' copyright exception;

3) This work has not been submitted for any degree or professional qualification except as specified.

Edinburgh, December 2017

**Emily Weiss**

## Abstract

This thesis looks at risk factors for multiple sclerosis (MS), a chronic, degenerative autoimmune disease which is usually diagnosed between the ages of 20 and 50 years. It is estimated to affect over 100,000 people in the UK. The research setting was Orkney and Shetland, two archipelagos situated north of mainland Scotland, and both of which have very high MS prevalence as do other countries at high latitudes. I examine genetic and environmental risk factors in Orkney and Shetland using multiple methods over four studies. I also review the vitamin D and UV exposure literatures as these are risk factors pertinent to MS in Orkney and Shetland.

After devoting three chapters to introducing the purpose of the thesis, MS, and Orkney and Shetland, in the fourth chapter, I aim to establish whether the birthplace of cases show any spatial, temporal, or spatiotemporal clustering. Evidence of these kinds of clustering may indicate that there are environmental risk factors present in some areas or that were present over particular periods, which raise risk of developing MS. Although I find statistically significant temporal, spatial, and spatiotemporal clustering in Orkney, and a spatial cluster in Shetland, for multiple reasons these results need to be interpreted with caution. I conclude that the clusters are very likely to be artefacts. Furthermore, there are multiple possible alternative explanations for such clusters that could not be explored by the available data.

Chapter 5 examines the heritability of MS in Orkney and Shetland to estimate the proportion of phenotypic variance attributable to additive genetic effects. I also look at the birthplaces of ancestors of cases and controls to see if any locations contribute a greater amount of ancestral DNA to the gene pool of modern MS cases, which I term 'genetic clustering'. In Orkney I obtained a heritability estimate of 0.36 (95% CI -0.26, 0.98); in Shetland this estimate was 0.20 (95% CI -1.88, 2.28). These modest estimates are consistent with the heritability literature. The genetic clustering

analyses highlight two Orkney registration districts, Kirkwall and Westray, which earlier studies identified as areas of MS clustering. I also identify three Shetland registration districts, however these locations had not shown any evidence of clustering in earlier studies. Again, I advise caution in interpreting results, particularly as all the error bars across registration districts overlap.

Chapter 6 presents a scoping review to map the literature and identify evidence of an association between vitamin D and UV exposure with MS. In methodically searching the literature, I identify a large and heterogeneous evidence base comprising multiple observational, intervention, and genetic studies. Overall, many studies support an association between vitamin D deficiency and MS. There is also evidence for an association between UV exposure and MS, although UV exposure is considerably less explored than vitamin D. I finally identify gaps in the literature and make suggestions for future research.

In Chapter 7 I aim to compare vitamin D levels in Orkney and mainland Scotland, and establish the determinants of vitamin D status in Orkney. I firstly compare mean vitamin D and prevalence of deficiency in cross-sectional data from studies in Orkney and mainland Scotland. I secondly use multivariable regression to identify factors associated with vitamin D levels in Orkney. I find that mean (standard deviation) vitamin D is significantly higher in Orkney compared to mainland Scotland (35.3 (18.0) and 31.7 (21.2), respectively), and prevalence of severe deficiency is lower in Orkney (6.6% to 16.2% $p = 1.1 \times 10^{-15}$). Factors associated with higher vitamin D in Orkney include older age, farming occupations and foreign holidays. I conclude that although mean vitamin D levels are higher in Orkney compared to mainland Scotland, there is substantial variation within the Orkney population which may influence MS risk.

Chapter 8 examines the correlates and determinants of UVB exposure in Shetland. I firstly construct correlation matrices to visualise how 1) personal characteristics such as sex, occupation, and skin type, 2) physical activity, and 3) body weight and fat, correlate with UVB exposure. I then use multivariable regression to identify factors associated with UVB exposure in Shetland. I run two multivariable models. The first includes the full sample size where activity data were measured by questionnaires. The second includes both questionnaire physical activity data and step-count data from pedometers, however as only a subset of participants had been supplied with pedometers, this analysis comprises a smaller sample size. I find that the amount of skin exposed was most strongly correlated with UVB exposure. Step count and activity minutes were also moderately positively correlated, and indoor occupations moderately negatively correlated, with UVB exposure. The regression analysis using the full sample with questionnaire activity data found that factors associated with greater UVB exposure were age and ambient UVB, while working indoors was significantly associated with lower UVB exposure. The model including the pedometer data found that found that age, total steps, and the amount of ambient UVB were significantly associated with greater UVB exposure. I conclude that atmospheric conditions, working outdoors and older age are important factors in UVB exposure in Shetland. It remains to be seen how UVB exposure translates to vitamin D levels in Shetland.

I found evidence for environmental and genetic risk factors for MS in Orkney and Shetland. The two environmental risk factors, vitamin D deficiency and reduced UV exposure, are more likely to affect the younger population who are still within their lifetime risk of developing MS.

## Lay summary

Multiple sclerosis (MS) is a condition that results in progressive disability, is usually diagnosed between the ages of 20 and 50 years, and is thought to affect over 100,000 people in the UK. The islands of Orkney and Shetland are the worst affected. The reasons why so many people in Orkney and Shetland have MS is not clear, and so I set out to understand why people in these islands are at increased risk. To do so I first examined whether people who were born in particular places or times were more likely to develop MS later in life. This can help to identify features in the environment that may be related to an increased risk of MS. I found that people born in some places, and during some periods of time, appeared to be at greater risk. However, there are many reasons for this pattern, including problems with the data which may have incorrectly led to these results, and therefore I am cautious about drawing any conclusions from it. I then looked at possible genetic risk factors. I found that in Orkney, a little over one-third of differences in MS susceptibility could be attributed to genetic differences among individuals. In Shetland, genetic differences were responsible for one-fifth of differences in MS susceptibility. These results are similar to those found in studies of MS carried out elsewhere. The results mean that although genes are involved in MS, there must also be other elements that lead to the development of MS. I then looked at whether the ancestors of people with MS were more often born in the same place than ancestors of people without MS. I found three Shetland and two Orkney birthplaces where the ancestors of people with MS appeared to be more frequently born. However, again, multiple alternative explanations for this pattern need to be ruled out before we can conclude that genes are responsible for these findings.

In Chapter 6 I identified studies that examined whether low levels of vitamin D in the blood or too little sunshine are associated with a greater risk of MS or worse disease progression. Many studies that we found showed that low levels of vitamin D

and reduced sunshine exposure were present in people with MS, and were sometimes present when disease progression was worse.

Finally, I conducted two studies, one that examined vitamin D levels in Orkney, and the other that explored UV exposure in Shetland. The first study found that vitamin D levels in Orkney were higher than in mainland Scotland. I also found that older people, farmers, and people who take foreign holidays had higher vitamin D levels in Orkney. In the second study we found that older people, physical exercise, working outdoors, and the weather contributed to more exposure to sunshine in Shetland. Younger people – who are still within the ages at risk of being diagnosed with MS – are therefore more likely to have low vitamin D and low sunshine exposure than older people. These studies showed that genes contribute to the risk of MS. Younger people often have lower levels of vitamin D and sunshine exposure. Both of these factors may contribute to MS in Orkney and Shetland.

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## Abbreviations

| | | |
|---|---|---|
| 25(OH)D | - | 25-hydroxyvitamin D |
| BMI | - | Body mass index |
| CDMS | - | Clinically definite multiple sclerosis |
| CIS | - | Clinically isolated syndrome |
| CNS | - | Central nervous system |
| CSF | - | Cerebrospinal fluid |
| DBP | - | Vitamin D binding protein |
| EAE | - | Experimental autoimmune encephalomyelitis |
| EBV | - | Epstein-Barr virus |
| GWAS | - | Genome-wide association study |
| $h^2$ | - | Narrow-sense heritability |
| HLA | - | Human leukocyte antigen |
| IgG | - | Immunoglobulin G |
| IM | - | Infectious mononucleosis |
| IPAQ | - | International Physical Activity Questionnaire |
| MED | - | Minimal erythema dose |
| MET | - | Metabolic equivalent of task |
| MR | - | Mendelian randomisation |
| MRI | - | Magnetic resonance imaging |
| MS | - | Multiple sclerosis |
| NIMS | - | Northern Isles Multiple Sclerosis Study |
| NMO | - | Neuromyelitis optica |
| ON | - | Optic neuritis |
| OR | - | Odds ratio |
| ORCADES | - | Orkney Complex Disease Study |
| PA | - | Physical activity |
| PCA | - | Principal components analysis |
| PPMS | - | Primary progressive MS |
| RCT | - | Randomised controlled trial |
| RRMS | - | Relapsing-remitting multiple sclerosis |
| SED | - | Standard erythema dose |
| SES | - | Socioeconomic status |
| SNP | - | Single nucleotide polymorphism |
| SOCCS | - | Scottish Colorectal Cancer Study |
| SPMS | - | Secondary progressive multiple sclerosis |
| SZA | - | Solar zenith angle |
| TEMIS | - | Tropospheric Emissions Monitoring Internet Service |
| UV | - | Ultraviolet |
| UVB | - | Ultraviolet B |
| UVR | - | Ultraviolet radiation |
| VDR | - | Vitamin D receptor |
| VIKING | - | Viking Health Study Shetland |

# CHAPTER 1. INTRODUCTION

Research over the past sixty years has consistently identified a high prevalence of multiple sclerosis (MS) in Orkney and Shetland. The most recent study, conducted in 2012, found that Orkney had the highest MS prevalence in the world, and Shetland was close behind (Visser et al., 2012). Despite advances in our understanding of the pathology of MS and its global epidemiology, the reasons for the high prevalence in Orkney and Shetland remain unclear. This ambiguity is troubling, because the identification of risk factors may help to explain why the residents of these islands are more frequently affected by MS, and may also identify ways in which behaviours could be modified to reduce risk. To address this problem, this thesis explores two avenues of research. The first concentrates on the genetic involvement in MS in Orkney and Shetland. The second focusses on two environmental risk factors: vitamin D deficiency and reduced ultraviolet (UV) exposure from sunlight.

Two background chapters follow this introduction. Chapter 2 provides a detailed overview of MS, including the history of the disease, the development of diagnostic criteria, an overview of environmental and genetic risks, and the global and UK epidemiology of MS. Chapter 3 introduces the research setting of Orkney and Shetland. I discuss two qualities of the research setting in detail. Firstly, I give an overview of the high-latitude geographical location of Orkney and Shetland, and their characteristic inclement weather, both of which can impact on UV exposure and vitamin D levels in the population. Secondly, I describe the population histories from the time of their first settlement to today, covering archaeological evidence as well as historical and recent emigration and immigration. Despite the fluctuations in population, I explain that Orkney and Shetland are genetic isolates, shown through distinct genetic structures that are different from the rest of the UK, and different from

each other. Both genetic isolation, and the geographical location, are qualities that may increase MS risk in these islands.

The high-latitude geographical location of Orkney and Shetland, alongside their often-inclement weather, means that sunshine is in short supply. Judicious exposure to UVB radiation from sunshine appears to be vital to human health, partly because it enables us to synthesise vitamin D from cutaneous cholesterol, and partly because it affects our immune systems independent of vitamin D. Inadequate UVB exposure and vitamin D deficiency have long been associated with MS. Additionally, there is a considerable literature that has sought to identify apparent clusters of MS to discover and attribute possible environmental causes for disease. Such endeavours have proved largely unsuccessful in identifying any causal agents, however it is possible that unidentified risks may be present in Orkney and Shetland which contribute to the high prevalence. Furthermore, genetic isolation can have adverse consequences for health. For example, it is possible that an as-yet unidentified genetic risk for MS could be found within Orkney and Shetland, or that common genetic variants which may collectively act to increase MS risk may present more frequently in this population. Potential genetic and environmental risks needed investigating in Orkney and Shetland, and were the aims addressed throughout this thesis. The questions that I sought to answer are as follows:

1) Are the people born within some parishes or isles more frequently affected by MS than would be expected by chance?
2) Do genes contribute a greater amount to the risk of MS in Orkney and Shetland than is observed elsewhere?
3) Is vitamin D deficiency a potential risk factor for MS in Orkney that could help explain the high prevalence?

4) Is inadequate UVB exposure a potential risk factor for MS in Shetland that could help explain the high prevalence?

Chapter 4 is devoted to addressing the first question. I explore the pattern of MS in Orkney and Shetland using temporal, spatial, and spatiotemporal clustering analyses. For these, I used participants' birthdates and birthplaces. It has previously been hypothesised that MS risk can be increased by factors from or before birth, or in early childhood. Therefore, clustering by birthplace may identify the presence of early-life risk factors. However, the limitations of our data were such that, although statistically speaking it appeared that clusters were present, there was no way to meaningfully interpret them. Furthermore, the quality of the data was such that it remains questionable whether the statistically significant presence of clusters actually denotes the presence of true clusters. Despite all efforts to be as rigorous as possible regarding definitions of cases and controls, significant problems beyond our control remain, and there are multiple other reasons to explain apparent aggregations of disease.

In Chapter 5 I explore the genetic contribution to MS in the Northern Isles, and how estimates of heritability in Orkney and Shetland compare with what is expected in the literature. As MS is a dichotomous trait (one either has it or does not), we calculated the heritability of liability to disease. This type of heritability estimate is based on the assumption that each individual has an underlying disease risk which is normally-distributed, called the liability, and is thought to determine an individual's probability of developing disease. I obtained modest estimates of the heritability to liability of MS from both Shetland and Orkney, suggesting that 20% (95% CI -188.0 to 228.0) (Shetland) and 36% (95% CI -26.0 to 98.0) (Orkney) of the variation in liability to MS between individuals is owing to additive genetic effects. However, the confidence

intervals are large and cross zero, probably resulting from overall small sample sizes and few related cases. I also explore possible genetic clustering of MS, using the strong genetic structuring in Orkney and Shetland by parish or isle as a proxy for genetic relatedness. To do so, I used the places of birth of ancestors of cases who had been born circa 1890, and weighted them by relatedness (for example, grandparents would contribute 0.25, great grandparents 0.125 etc.). This gave a genetic score for each district which denoted how much of a contribution each district made to the modern MS gene pool. I repeated the process with controls, and plotted all scores. The distribution across all registration districts were similar, and therefore no district appears to contribute more to the modern gene pool of MS than any other.

The second half of the thesis is devoted to exploring the environmental risk factors of vitamin D deficiency and reduced exposure to UV from sunlight. To begin, Chapter 6 presents a scoping review of the literature concerning MS, vitamin D deficiency, and UV exposure. The scoping review methodology enables a way to aggregate all relevant literature and present findings in a broad, narrative account. I divided the identified literature into three main areas: observational studies, intervention studies, and genetic studies, and discuss patterns in findings, possible reasons for discrepancies, and gaps in the literature.

In Chapter 7 I explore vitamin D levels in a cohort of Orcadians, and compare them to a cohort from the mainland of Scotland. Conversely to what we had anticipated, I found that Orcadians had higher mean vitamin D levels than people in mainland Scotland; furthermore, older people in Orkney had higher mean vitamin D levels than younger people. Further explorations of the data revealed that older people were more likely to work in traditional, outdoors occupation. Additionally, older people were more likely to take holidays abroad. Both these factors enable maximisation of sunshine

within Orkney and abroad, compared to indoor workers who largely remain within Orkney.

Chapter 8 is concerned with identifying the determinants of individual UV exposure in Shetland. I began by identifying correlations between individual UV exposure and a range of lifestyle variables. I followed this with two multivariable analyses, both with individual UV exposure as the outcome, however Model 1 included questionnaire physical activity data, and Model 2 included pedometer activity data as well as the questionnaire physical activity. Both models yielded slightly different results. However, both models were in agreement that older age and greater ambient UV were associated with higher individual UV exposure.

Finally, Chapter 9 contains a summary of the main findings of this thesis, how they fit into the existing literature, and their limitations. I conclude by addressing the implications of these findings. Particularly prominent is the need to firmly establish whether there is a causal connection between vitamin D deficiency and UV exposure with MS, to support efforts to modify these risk factors in Orkney and Shetland.

# CHAPTER 2. MULTIPLE SCLEROSIS: HISTORY, CLASSIFICATION, PATHOLOGY, AND EPIDEMIOLOGY

In this chapter I give a broad introduction to multiple sclerosis (MS) and the evolution of our understanding of the disease. Beginning with a brief history of MS, I draw on early examples from mythology and historical literature, leading to how MS was originally recognised and framed as a disease. I then give a simplified overview of our current understanding of the molecular processes that underlie the pathology, and describe how these processes translate into symptomatic disease. This leads me to outline the development of the main sets of criteria that have been, and are now, used to diagnose this complex disease. In so doing, I also present the challenges involved in comparing older studies which use early criteria, with newer studies which use updated and current criteria, and how comparisons of studies in different geographical regions can present difficulties. Finally, I describe the epidemiology of MS, giving an overview of the main environmental and genetic risk factors, and the global and UK prevalence of MS.

## 2.1 History of MS

Before MS had been framed as a disease, details of mysterious illnesses with multiple transient neurological symptoms had been recorded for centuries in mythologies, genealogies, diaries, personal correspondence, and medical notes. Looking at such cases through the prism of modern medicine, it is possible to hypothesise that at least some of these unexplained illnesses may be attributable to MS; at the very least, MS would likely be highlighted as a possibility on a list of differential diagnoses.

### 2.1.1 Icelandic Sagas

One of the earliest recorded 'possible MS' cases is that of Halldora, in the Saga of Thorlak, one of the Icelandic Biskopa Sögur or Bishops' Sagas. Halldora was a young woman on the Vestmann Islands, who suffered from intermittent paresis (weakness or

partial paralysis) between 1193 and 1198. After three years confined to bed, the miraculous power of Bishop Thorlak, who had died in 1193, was called upon to cure Halldora. The Bishop appeared to a woman in a dream with instructions that Halldora must go to Skálholt if she wished to be cured. She was carried to Skálholt and arrived in time for the church festival where Bishop Paul requested that the congregation pray for her. She grew a little better each day that she was in Skálholt; the day following the festival's end she went to the Althing and offered up a gold ring and shortly after was completely cured (Holmøy, 2006).

A further tale appears in the same saga of Bishop Thorlak, concerning a young woman named Halla who lived more than 100 years after Halldora, with her illness occurring between 1293 and 1323. Halla was said to have fallen ill on a Saturday, suddenly losing sight in both her eyes and developing bilateral ptosis (drooping eyelids). The following day she lost her speech. She is said to have made a vow to "almighty God for cure and Bishop Thorlak for intercession" that she would walk to Skálholt, fasting on bread and water, and praying before Thorlak's mass. On the third day, it is said that a "candle wick was put around her head", following which she regained the sight in one eye and was able to open both eyes. Within a week of the original illness she regained her speech, and within two weeks the sight in her second eye was restored (Poser, 1994).

The paucity of detail within both stories makes it difficult to accept a diagnosis of MS without some scepticism, particularly as these stories originate from an era in which illness and disease were little understood and powerful emotions were associated with fervent belief (Poser, 1994). The symptoms described in both stories are not incompatible with MS, however Halldora's three year paresis is unusually long (Holmøy, 2006). Additionally, while some of Halla's symptoms, such as bilateral loss of

vision, bilateral ptosis, and loss of speech are rare events in MS they are not unheard of. Hysteria has been considered the most likely explanation of their symptoms by readers of the untranslated sagas (Compston et al., 2006a), however others argue that the slow improvements recorded for both women make hysteria a questionable explanation, as any such symptoms would be likely to resolve swiftly (Poser, 1994). The cure of Halldora was one of the miracles for which Thorlak was canonised, and it is possible that the symptoms described were exaggerated to deepen the miraculous nature of the cure (Holmøy, 2006). Regardless of diagnoses, what these stories do show is that temporary paralysis was recognised in medieval Iceland (Holmøy 2006).

### 2.1.2 Lidwina of Schiedam

Lidwina the Virgin, born in Schiedam, Holland, in 1380, is another early speculative example of "possible" MS. Following an illness in the winter of 1395-96 from which she was slow to recover, she went ice-skating where she fell, and broke ribs on her right side. As the injury was healing she had difficulty walking and is described as having an intense pain in her teeth. Over the next years she lost sight in one eye, was sensitive to light, had paralysis in her right arm and pain in her right shoulder. She lost sensation and developed sores, and eventually had difficulty swallowing, became so weak she was unable to walk, and developed contractures of her limbs (Murray, 2004; Huysmans and Hastings, 1923).

However, like Halla and Halldora, Lidwina was fervently religious. She accepted her pain with an apparently pious joy and latterly took steps to increase her discomfort by swapping her bed for wooden planks and hay, and wearing a belt of horsehair to rub her remaining healthy skin raw (Huysmans and Hastings, 1923). A mythology grew up around her in her own lifetime; her sores and ulcers were said to emit a perfumed scent; she cried tears of blood; she turned a pitcher of beer into an aromatic potion. Further, her darkened room was said to glow – like fire – from visitations of angels and

saints. During the final three decades of her illness she was said to have experienced several such apparitions, and had visions of participating in the Passion of Christ following which her stigmata appeared. Through the later stages of her illness she was reportedly unable to tolerate any food other than consecrated Holy Communion wafers. Lidwina died in 1433 at the age of 53, her corpse apparently resembling her 17-year old self and bearing no signs of her illness (Huysmans and Hastings, 1923). She was canonised in 1890, and is the patron saint of the chronically ill and (rather bizarrely) of ice-skating.

Although many of her reported symptoms are compatible with an MS-like illness, like Halla and Halldora religious fervency and hysteria cannot be disregarded as reasonable explanations. Murray further notes that elements of histrionic behaviour and self-mutilation need to be considered (Murray, 2004). Although contemporary documents are in existence charting the life of this unfortunate woman, the mythology that had already built up around her must undermine their accuracy.

### 2.1.3 Augustus d'Esté

Augustus d'Esté, born in 1794, was the son of Prince Augustus Frederick, Duke of Sussex and Lady Augusta Murray. Through his father he was also the grandson of King George III. However, the marriage of Prince Augustus and Lady Augusta was declared invalid by the King shortly after taking place, as he had never given the union his royal consent. Augustus d'Esté was thereby rendered illegitimate (Este, 1832).

Harrow-educated, and rising to the rank of Lieutenant Colonel in the VIIth Royal Fusiliers in 1812, Augustus d'Esté had an unremarkable childhood and, it appears, a dissolute and rather boorish young adulthood (Firth, 1948). His greatest legacy, however, is his diary and gentleman's almanac in which he recorded his descent

into an unexplained illness that lasted from 1822 until his death in 1848, and the treatments that he underwent in the hope of a cure (Firth, 1941).

His symptoms first presented with bilateral optic neuritis (ON), which he attributed to exerting extreme effort not to cry at the funeral of a close friend. Over the next twenty years, further symptoms developed including diplopia (double vision), paraparesis (partial or mild paralysis of the lower limbs), anal incontinence and impotence, lack of coordination, weakness, sensory changes and cognitive impairment (Landtblom et al., 2010; Compston et al., 2006a). A relapsing-remitting course is described in the first years of disease, with complete recovery following the first two episodes and only partial recovery following the third (Compston et al., 2006a). He consulted the top physicians of the day, and underwent numerous treatments in the UK and Europe including bloodletting, leeches, emetics, a diet of beef steak and sweet wine, having his legs and back rubbed with brushes, spa therapies, tincture of Spanish fly, application of heat as hot as he could bear, herb and flower preparations, and daily showers. He was also prescribed and tried mercury, electricity, horse-riding, and taking the sea air in Brighton (Murray, 2004). Following periods of improvement, secondary progressive disease began between 1829 and 1839. In 1847 he began timing his daily walks around his apartment to within a fraction of a minute and recording them in his almanac, charting his decline and occasional achievements (January 17th 1848: "Not bad! I walk, this day in my room for 45¾" (Compston et al., 2006a)). He died in 1848 having been confined to a wheelchair for his last years (Landtblom et al., 2010). It is widely accepted that the diary of Augustus d'Esté provides the first comprehensive account of definite MS (Murray, 2004; Compston et al., 2006a).

### 2.1.4 W N P Barbellion

Before the discovery of Augustus d'Esté's diary in 1942, the most complete account of MS was published in the Journal of a Disappointed Man by W N P Barbellion,

the pseudonym of Bruce Frederick Cummings, who was born in 1889 in Barnstaple, Devon (Compston et al., 2006a). In April 1913 Barbellion noted with anxiety that he had some paralysis down his right side. His speech was affected, and he was placed on two months' sick leave. Ocular disturbances, giddiness, fatigue, and nerve pains affected him over the coming months and years, and although he consulted a number of physicians, he only discovered his diagnosis after attempting to enlist in the army. Opening a sealed letter from his physician to the recruiting army Medical Officer, he saw his condition named: disseminated sclerosis (Barbellion, 1919).

Cummings married after symptom onset but before he knew his diagnosis. His fiancée had been told and 'had married him for love, nevertheless, against every friendly counsel, the Doctor's included' (Barbellion, 1919). They had two daughters, and Cummings followed a successful career as an entomologist at the Natural History Museum in London. He died in 1919 at the age of 30, having announced the death of Barbellion some two years previously on publication of the diary (Barbellion, 1919).

Transient neurological illness that worsens over time has therefore been described for many years, with an interesting history of how those affected lived with and managed their symptoms. However, whilst the disparate collection of symptoms that may indicate MS had been noted and described before official recognition of the disease, it was not until the mid- to late-nineteenth century that the condition was encapsulated, described, and named. The next section describes how MS was first identified, moving on to give an overview of some of the key molecular changes that occur in the immune system, how these changes can lead to symptomatic disease, and how such different symptoms may be classified.

## 2.2 Classification of MS

In 1868, the Parisian neurologist, Jean Martin Charcot, identified a triad of features which often presented together: intention tremor (involuntary movement of the hands associated with deliberate movements), nystagmus (involuntary movement of the eyes) and scanning speech (disruption of normal speech pattern characterised by long pauses). Following the dissection of the brain of a deceased patient exhibiting these three symptoms, Charcot identified the plaques associated with MS, calling it 'la sclérose en plaques' (Murray, 2004). Since that original recognition of the characteristic plaques, our understanding of MS, and the molecular changes that lead to symptomatic disease, has increased.

### 2.2.1 Molecular involvement

MS is a chronic, autoimmune, degenerative disease, where the body's immune system attacks myelin, the fatty insulation of neurons which facilitates transmission of nerve impulses. These attacks lead to damage and destruction of the myelin, resulting in the formation of sclerotic plaques (abnormal hardening of the tissue), and the onset of symptomatic disease (Compston and Coles, 2008). However, these plaques are the culmination of a process involving inflammation, demyelination, remyelination, depletion of myelin-producing oligodendrocytes and scar-forming astrocytes, and degeneration of neurons and axons (Compston and Coles, 2008). Although such complex processes are beyond the scope of this chapter to discuss in detail, it is clear that multiple cells are involved in each stage of disease.

It is thought that the disease is initiated when autoreactive lymphocytes, namely T and B cells, migrate across the blood-brain barrier. In people with MS, defective regulatory cells, such as $T_{reg}$ cells, do not suppress effector T cells, and therefore fail to shut down immune responses. Cells including T and B cells, plasma cells, and macrophages, accrue, and pro-inflammatory cytokines boost the immune

response by recruiting microglial cells (Compston and Coles, 2008). The microglia are innate immune cells, which activate when they detect pro-inflammatory factors. They then release cytotoxic elements, such as tumour necrosis factor, which aims to protect the body by destroying harmful invading pathogens. In MS, this microglial process may be critical in inflicting oligodendrocyte injury and degenerating axons (Correale and Farez, 2015; Lull and Block, 2010), which leads to increased grey and white matter atrophy (Dendrou et al., 2015). Astrocytes respond to chronic injury causing gliosis, a scar composed primarily of astrocytes although it can contain other glial cells including oligodendrocytes and fibromeningeal cells. The gliosis can effectively prevent repair and lead ultimately to permanent sclerotic plaques (Compston and Coles, 2008; Correale and Farez, 2015). These areas of damage in the central nervous system (CNS) are visible on MRI scans, and are one of the signs that MS is probably present.

Clinical tests can detect other molecular changes. Cerebrospinal fluid (CSF) examination from people with MS tends to contain elevated immunoglobulin G (IgG) levels, which can be visualised as oligoclonal bands. Where oligoclonal bands are observed in CSF but not in serum, or where additional bands are observed in CSF that are not observed in serum, then there is clear evidence that the IgG is being produced within the CNS. As IgG is produced by sclerotic plaques, the presence of IgG in the CSF implies that there are active immunological processes in the CNS (Miller et al., 2006). Such increased understanding of these molecular processes has aided in the diagnosis of MS, and has also led to the identification of molecules that can be therapeutically targeted (Noseworthy et al., 2006).

### 2.2.2 Physical symptoms

The physical symptoms that result from these molecular processes are usually the first sign to the patient that something is amiss. The plaques that form following the demyelination process disrupt nerve signals resulting in a poor connection between the

brain and muscles, and ultimately leads to symptomatic disease (Confavreux and Compston, 2006).

MS can manifest with a wide variety of different symptoms depending upon the location of the scleroses. In the cerebrum, cognitive impairments and depression can ensue; in the optic nerve, unilateral loss of vision; in the cerebellum and cerebellar pathways, tremor, and clumsiness including poor balance; in the brainstem, vertigo, difficulty swallowing, diplopia, oscillopsia (where objects in the field of vision appear to oscillate), and impaired speech; in the spinal cord, weakness, spasticity, bladder dysfunction; and in other areas, pain, fatigue, and sensitivity to temperature can result (Compston and Coles, 2008). Alongside these manifold symptoms, several different presentations of MS can occur. However, the current classification guidelines split MS into two core phenotypes: relapsing-remitting, and progressive disease. Each phenotype includes a spectrum of characteristics which are further sub-divided into active or inactive disease (Lublin, 2014).

### 2.2.3 Relapsing-remitting disease

Relapsing-remitting MS (RRMS) is the most common type of MS; symptoms develop over the course of a few days and then resolve either partially or fully over a few weeks or months. As RRMS progresses recovery from relapses becomes less complete and the condition gradually worsens; this is termed secondary progressive MS (SPMS). Most people with RRMS will develop SPMS within twenty years of disease onset (MS, 2015).

Clinically isolated syndromes (CIS), such as optic neuritis (where a lesion appears on the optic nerve leading to disturbed vision) or transverse myelitis (where a section of the spinal cord becomes inflamed), can be early symptoms of MS. According to the most current criteria for defining MS subtype, such CIS can be classified as

inactive if there is no evidence of disease exacerbation following an episode, and classified as active CIS if there is evidence of disease exacerbation following an episode. Active CIS may then fulfil criteria to be labelled RRMS (Polman et al., 2011). RRMS is also characterised as active or inactive, although yearly medical assessment for disease activity is recommended (Lublin, 2014).

### 2.2.4 Progressive disease

Primary progressive MS (PPMS) is defined as MS that presents with symptoms that worsen over time: there are no periods of remission and likewise, there are no sudden periods of deterioration. It is instead a slow and steady decline, although there may be periods of slight improvement (MS, 2015).

By the most current definition, progressive disease encompasses both primary and secondary progression, and further relates disease progress to the amount of disability. There are four sub-divisions: active disease with progression, where the individual has had an attack and is worsening; active disease without progression, where an individual has previously had an attack within a particular time frame; not active with progression, defined by yearly clinical evaluation of abilities, and not active and without progression, which summarises stable disease (Lublin, 2014).

There are therefore many physical symptoms associated with MS, and there is an increasing knowledge of the molecular processes involved in the pathology since Charcot's original recognition of MS to today. However, despite such breakthroughs, diagnosing MS is a highly complex process. One key ongoing debate is whether MS has a range of different presentations and phenotypes which stem from a single cause, or whether many causes leading to different disease mechanisms result in a set of clinical signs and symptoms which fall within the spectrum of a single disorder (Lassmann et al., 2006). Difficulties arise as no clinical tests can definitively diagnose MS. A positive

result simply indicates that there is immune activity: other diseases of the CNS can produce the same clinical signs. Additionally, the myriad symptoms that can appear do not, on their own, definitively indicate MS. However, as our knowledge base has increased, a number of sets of criteria to aid in the diagnosis of MS have been developed, refined, and revised to try to narrow down those characteristics that make anything other than MS unlikely. I present here a brief overview of the development of the main sets of criteria to those that are used today, and outline the challenges involved in comparing studies that use different diagnostic criteria.

### 2.2.5 Allison and Millar criteria

The Allison and Millar criteria were probably the most successful of the early attempts to create a clear diagnostic scheme against which to define MS. It divided cases into 'early', 'probable', and 'possible' disseminated sclerosis (Allison and Millar, 1954). Early disseminated sclerosis comprised people with a history of relapsing-remitting symptoms, but with no or few clinical signs. This category is however not acceptable by the standards of modern diagnostic criteria (Poser and Brinar, 2004). Probable disseminated sclerosis included people with physical signs of MS, usually with some disability, and presenting with a history equating to a modern diagnosis of RRMS or SPMS (Poser and Brinar, 2004). Possible disseminated sclerosis comprised people with either unchanging or chronically progressive symptoms for which no other explanation had been found, equating most often to a modern diagnosis of PPMS (Allison and Millar, 1954; Price, 2009). The vagueness of such criteria has however been highlighted as being of little diagnostic value (Poser and Brinar, 2004). Moreover, despite the authors' warning that the "criteria should be used only by experienced neurologists, certainly not by surrogates carrying on epidemiological surveys" (Allison and Millar, 1954), up until the 1980s many MS surveys used some adaptation of the Allison and Millar criteria (Compston et al., 2006a).

### 2.2.6 Schumacher criteria

The Schumacher criteria were developed in 1961 and published in 1965 to meet a growing need for clearly defined diagnostic guidelines to which MS researchers, investigators, writers, and editors of scientific journals could adhere. Such comprehensive guidelines were anticipated to provide consistency in research and to aid collaboration between different institutions (Schumacher et al., 1965; Poser and Brinar, 2004). These criteria required objective signs of CNS dysfunction, a history of the involvement of two or more areas of the CNS with two or more episodes each lasting at least 24 hours and occurring at least one month apart, and progression of the disease over at least six months. Predominantly white matter involvement and onset age between 10 and 50 years were also listed. Like the Allison and Millar criteria, there should be no other better explanation for symptoms, based upon the examining clinician's experience and expertise (Schumacher et al., 1965). An update of the Schumacher criteria in 1980 were the first to include testing for oligoclonal bands in CSF (Poser and Brinar, 2004).

Whilst it was clearly noted that the Schumacher criteria may be subject to some false positive and false negative findings, it was nevertheless advised that those who met all criteria should be categorised as having definite MS and accepted into studies. However, owing to a lack tests that indisputably indicate the presence of MS it was highlighted that this 'definite' category should be considered high probability of MS only, and not a definitive diagnosis. The proposed guidelines became the gold standard, widely used in clinical settings and epidemiological studies worldwide excepting East Asia, where the presentation of MS is often different. This point is discussed further below (see section 2.2.9) (Poser and Brinar, 2004).

### 2.2.7 Poser criteria

In 1965 Charles Poser wrote "many clinicians… insist that there is,… in diagnosing MS, an… almost mystic diagnostic item frequently referred to as the "feel" or the "smell" of the patient, and which can be best characterized by the almost classical, pontifical pronouncement: 'Don't ask me why I think this patient has MS, I just know!'" (Poser, 1965). Poser's 1965 study, conducted across 33 countries and involving 108 clinicians including MS experts and general neurologists, highlighted that the application of any set of criteria to autopsy-verified MS cases, patients without MS, and patients with MS and another CNS condition, led to a consistent diagnostic accuracy rate of two-thirds – although it was not always the same two thirds that were correctly identified.

Soon after, a number of diagnostic recommendations and new criteria were proposed, including those by Broman (Broman et al., 1965) who was the first to incorporate CSF examination before the significance of oligoclonal bands was discovered, and to clearly state the importance of dissemination of lesions in time and dissemination of lesions in space, two criteria which are central to MS diagnosis today (Poser and Brinar, 2004). Dissemination in time requires that abnormal activity in the CNS must be observed on at least two occasions; dissemination in space means that abnormal activity must affect at least two areas of the CNS, Other criteria were proposed by Poser, which never gained widespread acceptance (Poser, 1979), and several other researchers who all divided patients into some form of probable or possible MS (Poser and Brinar, 2004). However, in 1982 a panel led by Poser, including distinguished researchers in many aspects of MS, met in Washington DC with the aim of constructing a new set of clearly defined criteria incorporating laboratory evidence and reducing reliance on subjective assessment.

The Poser criteria, proposed in 1983, became the gold standard in MS diagnosis and remained in use until the end of the millennium (Poser et al., 1983). Dividing patients into 'clinically definite', 'laboratory-supported clinically definite', 'clinically probable' and 'laboratory-supported clinically probable' MS depending upon their history and evidence of disease, the Poser criteria required at least two attacks with either laboratory-supported (oligoclonal bands or increased IgG production in the CSF), or paraclinical (evoked potentials – to measure electrical impulses in response to stimulation of specific sensory pathways), or tissue imaging (such as CT scanning) evidence of other lesions (Poser et al., 1983). The Poser criteria did not however make accommodations for 'suspected' MS, as these individuals would not be acceptable for inclusion in research studies or clinical trials (Poser and Brinar, 2004; Poser et al., 1983). 'Suspected' MS was therefore assigned to all patients thought to have demyelinating disease but without clinical symptoms, signs or laboratory evidence for more than one lesion (Compston et al., 2006a).

### 2.2.8 McDonald Criteria

The Poser criteria developed into the McDonald criteria, which were first introduced in 2001 by a team led by Professor Ian McDonald, and reflected advances in technology and increased understanding of the disease (Fangerau et al., 2004). The McDonald criteria divide people into 'MS', 'possible MS' and 'not MS'. They have since been updated twice, in 2005 and 2010.

The McDonald criteria require evidence of CNS damage in space and in time, defined as damage to two parts of the CNS occurring at least 30 days apart, with attacks that last for at least 24 hours. The criteria also incorporates CSF analysis, visual evoked potentials (to test optic nerve transmission), and, possibly the biggest difference from the preceding criteria, MRI data (Polman et al., 2011). These MRI data enable an MS diagnosis to be given to patients who have experienced CIS suggestive of MS. As such, it

has been implied that the criteria are prognostic for future disease activity rather than diagnostic of MS, and will therefore identify individuals who would not have fulfilled earlier diagnostic criteria (Miller et al., 2008). Additionally, about 1% of people who fulfilled earlier criteria for MS are diagnosed as 'Not MS' under the McDonald criteria (Fox et al., 2004).

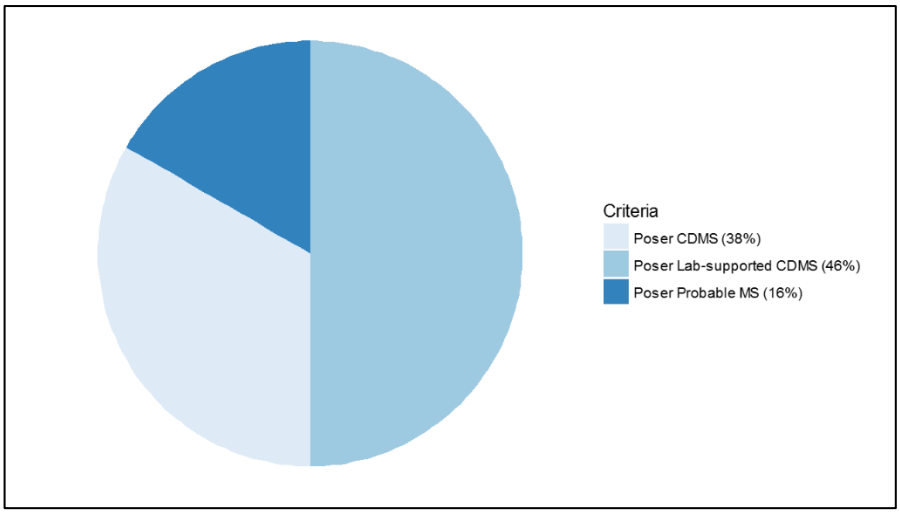
Nevertheless, soon after their introduction, the sensitivity (the probability that a test will find the disease among those who have the disease), and specificity (the probability that a test will find no disease among those who do not have the disease), of the McDonald criteria were shown to be high in comparison with the then-gold standard Poser criteria (Dalton et al., 2002; Tintoré et al., 2003; Swanton et al., 2007). Furthermore, the positive predictive value (the percentage of people who have a positive test result who actually have the disease) and the negative predictive value (the percentage of people with a negative test result who do not have the disease), of the McDonald criteria were both found to be high when the criteria were applied to CIS patients and followed up at different time points over three years (Dalton et al., 2002). These results indicate that the McDonald criteria's ability to diagnose MS from CIS leads to accurate early detection of disease.

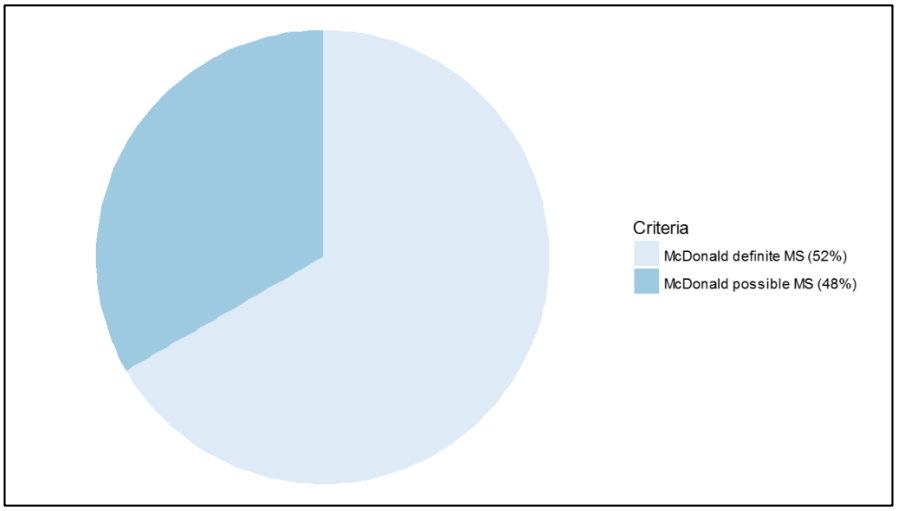
Whilst diagnostic accuracy is increasing with improved diagnostic criteria, it is however important to note that there is still no test that indisputably indicates the presence of MS. Rather, diagnosis is made by neurologists through building a clinical picture of each patient's signs, symptoms, disease course and investigative test results, and by ensuring that differential diagnoses are excluded (Miller et al., 2006). Despite Poser's earlier concerns, clinician experience and expertise remain a vital part of MS diagnosis.

### 2.2.9 Comparisons of studies across time and geographical regions

Diagnostic criteria have therefore developed to both meet changing needs and to reflect advances in technology and increased knowledge of MS. However, these changes complicate comparisons of older studies with new. The ways in which cases were defined differs between the Allison and Millar criteria and the Poser criteria, hindering direct comparisons. Such differences arise when surveys are restricted to categories of probable and early (Allison and Millar), and definite and probable (Poser) cases. Because the way in which possible and suspected cases are excluded differs between the two criteria, included cases meet different diagnostic standards and are not necessarily comparable (Compston et al., 2006a). Additionally, comparisons between McDonald and Poser studies are also complicated by the differing categorisation of cases. A study comparing diagnoses made by the Poser criteria and McDonald criteria found that whilst the McDonald criteria were able to diagnose MS more frequently than 'clinically definite MS' according to the Poser criteria, when the 'clinically definite' and 'laboratory-supported clinically definite' Poser categories were combined then the Poser criteria diagnosed MS more frequently (Figure 2.1) (Fangerau et al., 2004).

Figure 2.1 Pie charts of Poser- and McDonald-defined clinically definite MS (CDMS) and probable/possible MS




Criteria choice can also complicate comparisons of contemporaneous studies based in different geographical regions. There is an ongoing debate regarding possible heterogeneity of MS pathology, diagnostic criteria for which may be capturing diseases which should be considered distinct entities (Compston et al., 2006b; Lassmann et al., 2006). For example, comparisons of European Americans and African Americans with MS found differing presentations of MS, with significantly more neuromyelitis optica (NMO) (an inflammatory disease of the CNS with optic nerve and spinal cord involvement), and transverse myelitis (an inflamed section of spinal cord), observed in

the African American cohort. African heritage MS patients were further found to need ambulatory assistance more often than their European heritage counterparts suggesting more aggressive disease (Cree et al., 2004). The Latin American presentation of MS appears to comprise greater frequency of opticospinal involvement than observed in a European heritage MS population; NMO also presents more frequently (Aguirre-Cruz et al., 2011).

Likewise, the East Asian presentation of MS tends to include a greater frequency of NMO-like MS. Studies of Japanese MS patients have shown that the presentation tends to be opticospinal, and the lesions often destructive and necrotic (Tabira and Tateishi, 1981; Ikuta et al., 1981). Recent work in Japan has suggested that as between 50-60% of opticospinal MS patients have the specific immunoglobulin against NMO, NMO-IgG, then opticospinal MS may be the same entity as NMO rather than MS (Kira, 2008).

These differing presentations of MS in populations of non-European heritage mean that the criteria for diagnosing MS, which were developed against the 'conventional' type of MS observed in European populations and those of European heritage (Polman et al., 2011), do not necessarily capture phenotypes in other ethnic groups. This has led to the adaptation of criteria in some areas (Chong et al., 2009). Such adaptations do however mean that atypical forms of MS are less likely to be detected by unadapted criteria in countries where conventional MS predominates (Cogan et al., 2014). The most current McDonald criteria attempts to address these differing presentations and bring some uniformity to diagnoses worldwide, with recommendations made specifically for Latin American and East Asian populations, as well as paediatric patients (Polman et al., 2011).

These criteria are therefore clearly beneficial in assisting and guiding diagnosis and promoting diagnostic consistency, however the complexity of MS is still being understood and no one test can diagnose MS with certainty. The lack of direct comparability between different sets of criteria, and the different presentations of MS in different populations and ethnicities which some criteria may be less able to accurately detect, complicate the interpretation of studies across time and across geographical regions. There is however no doubt that the evolution of our understanding of MS, which has led to an evolution in diagnostic criteria, and an evolution in the classification of MS into different subtypes, is work still ongoing.

## 2.3 Epidemiology of MS

Our increasing understanding of MS biology, its molecular processes and symptomatic manifestations, has played a vital role in our ability to recognise, and treat, MS. However, the mechanism by which the inflammatory reaction is initially triggered is still unclear, and it is this uncertainty that this thesis sets out to explore in more detail by examining relevant risk factors in areas of extremely high prevalence. The field of epidemiology works at the population level, and seeks to understand the causes, and the distribution, of disease.

To round off this chapter about MS, it is important to introduce some of the epidemiological features of MS. These features include some of the more prominent MS risk factors, including genetic risk factors, and environmental risk factors, including smoking, Epstein-Barr virus (EBV) antibodies, vitamin D deficiency, and reduced UV radiation, alongside a simplified overview of the possible mechanisms by which these risk factors may work to increase MS risk. I then discuss how MS is measured at the population level, and illustrate the general global and UK distribution of disease with reference to some of the risk factors, before presenting concluding remarks.

### 2.3.1 Genetic risk factors

Despite the earlier-mentioned controversies, MS is widely considered to be a disease of multifactorial origin, involving a complex interplay of genetic and environmental risk factors (Compston and Confavreux, 2006). Genetic epidemiology focusses on identifying the genetic causes of disease, and on the combined effects of genetic and environmental determinants (Smith et al., 2011).

A genetic predisposition to MS has long been suspected, strongly supported by an increased frequency of MS which was noted in the families of affected individuals (Reynolds, 1904; Davenport, 1922; Mackay, 1950). Additionally, heritability studies, which allow a comparison of the relative importance of environmental and genetic risk factors, have clearly demonstrated a genetic component to disease and are discussed in more detail in Chapter 5.

The Human Leukocyte Antigen (*HLA*) genes show the greatest genetic risk for MS. The *HLA* genes encode a group of proteins, together called the HLA complex, which work in the immune system to help distinguish between the body's proteins and invading pathogens such as bacteria and viruses (NIH, 2017). The strongest genetic risk factor for MS is an *HLA* haplotype, *HLA-DRB1*15:01*. People who have two copies of this haplotype are at high risk, with an odds ratio (OR) exceeding 7; people with just one copy of the haplotype have an OR of between 3.5 and 5.0 (Oksenberg and McCauley, 2016). A further high-risk *DRB1* genotype has been identified, *HLA-DRB1*15/08* which confers an OR of 7.7 (95% CI=4.1-14.4) (Barcellos et al., 2006).

As technology progressed, the age of genome-wide association studies (GWAS), enabled the fast analysis of thousands of individuals and a large number of genome-wide markers, which represent every gene in the genome. To date, this GWAS approach has identified 110 genetic variants in 103 separate loci, excluding those found within

the *HLA* (Oksenberg and McCauley, 2016). However, all these identified risk loci have fairly modest effect sizes, with ORs of between 1.09 and 1.34 (International Multiple Sclerosis Genetics Consortium and Wellcome Trust Case Control Consortium 2, 2011; International Multiple Sclerosis Genetics Consortium, 2013). Taken together, the proportion of genetic variance accounting for disease risk explained by these genetic variants is about 30%; additionally, the number of risk variants that each individual with MS carries varies considerably (Oksenberg and McCauley, 2016). Furthermore, these risk variants are also fairly common within the population (Oksenberg and McCauley, 2016), however not everyone who carries such genetic risks will develop MS. Therefore, other risk factors must be necessary for MS to be triggered. This prerequisite is further reinforced by an apparently increasing MS prevalence over the past fifty years (Orton et al., 2006), which may be partially explained by improvements in diagnostic criteria (see sections 2.2.6-2.2.8 and 2.3.3). However, as the frequency and distribution of genetic risks are unlikely to have changed over this time, it is argued that increased or decreased exposure to an environmental factor or factors must be crucial in triggering MS onset (Oksenberg and McCauley, 2016).

### 2.3.2 Environmental risk factors

Although environmental risk factors appear to be necessary for MS to manifest, identifying any such triggers has proved difficult and has led to a wide variety of diverse theories, some of which will be touched upon in Chapter 4. However, those theories that have been most enduring include smoking, the presence of EBV antibodies and infectious mononucleosis (glandular fever), vitamin D deficiency, and inadequate UV exposure.

Smoking has shown strong dose-dependent associations with a substantial increase in MS risk (Riise et al., 2003; Hernán et al., 2001). Furthermore, smoking may also be a risk factor in the conversion of CIS to clinically-definite MS (Di Pauli et al.,

2008), and from RRMS to SPMS (Hernán et al., 2005). There is also some evidence that smoking may interact with specific *HLA* genes to increase MS risk (Hedström et al., 2011). In a large umbrella review of systematic reviews, smoking was found to be associated with MS risk, with an OR of 1.52 (95% CI 1.39-1.66) (Belbasis et al., 2015). Although it is not clear quite how smoking increases MS risk, a study from Sweden, where snuff is commonly used (Eriksen, 2015), showed that cigarette smoking, but not snuff use, was linked to increased MS risk. This finding suggests that the deleterious effects of tobacco come from inhaling the smoke (Hedström et al., 2009).

The same umbrella review also found significant associations between MS and IgG seropositivity to EBV, (OR 4.46, 95% CI 3.26-6.09) and infectious mononucleosis (IM) (OR 2.17, 95% CI 1.97-2.39) (Belbasis et al., 2015). EBV is a common herpes virus, and is the cause of IM. Most people carry the virus without being aware of it, and not everyone who has the virus becomes sick with IM. However, MS appears to be very rare in people who have not been infected with EBV (Ascherio and Munger, 2007). It is therefore unsurprising that the review found that both EBV and IM were associated with an increased risk of MS. Additionally, a history of IM further increases risk of MS (Ramagopalan et al., 2010b). People with high levels of these EBV antibodies are at an increased risk of MS compared to people with lower levels, and further, the relationship appears to be temporal, as EBV antibodies increase several years before the onset on MS symptoms (Ascherio et al., 2001; Levin et al., 2005; Sundström et al., 2004). It is interesting to note, too, that a high level of EBV nuclear antigens, a protein which results from EBV infection, appears to interact with smoking to increase MS risk (OR 1.7, 95% CI 1.1-2.6) (Simon et al., 2010a). The mechanism by which EBV increases MS risk is unclear, however one theory involves molecular mimicry, defined as the cross-recognition of foreign and self peptides (Wekerle and Hohlfeld, 2003). It has been shown that a T-cell receptor from an MS patient can recognise two different peptides.

The first is an *HLA-DRB1*1501*-restricted myelin basic protein, a constituent of myelin, and the second is an *HLA-DRB5*0101*-restricted EBV peptide (Lang et al., 2002). This mimicry between a peptide involved in MS, and an EBV peptide, may influence MS risk.

Perhaps more controversial are the risk factors of reduced UV exposure and vitamin D deficiency with MS risk, explorations of which form three chapters of this thesis and will therefore not be discussed in detail here. There is however a long history of the link between reduced UV exposure, vitamin D deficiency and MS (Acheson et al., 1960; Goldberg, 1974; Kurtzke, 1975). Furthermore, there are compelling arguments for the ways in which UV exposure and vitamin D may act both synergistically and independently to increase MS risk (Lucas et al., 2015; Lucas et al., 2011). Finally, some interesting interactions have been identified between genes that are associated with vitamin D and increased MS risk (Ramagopalan et al., 2009b; Ramagopalan et al., 2011), all of which strongly suggests a causal association. One of the first clues that vitamin D may have a role in MS was from the geographical distribution of MS, which is introduced in the next section.

Several enduring theories, with increasingly compelling evidence, therefore exist to explain how MS may be triggered by environmental risk factors. Although their mechanisms are still not fully understood, epidemiology has enabled the identification of such risk factors, and also the interaction of risk factors. In the next section I discuss the concept of prevalence, and give a brief outline of the global and UK epidemiology of MS, before concluding this chapter.

### 2.3.3 MS prevalence

MS is usually described in terms of prevalence: the number of existing cases at either a particular point in time (point prevalence) or within a certain period (period prevalence). Point prevalence is the more common measure of the two (Szklo and

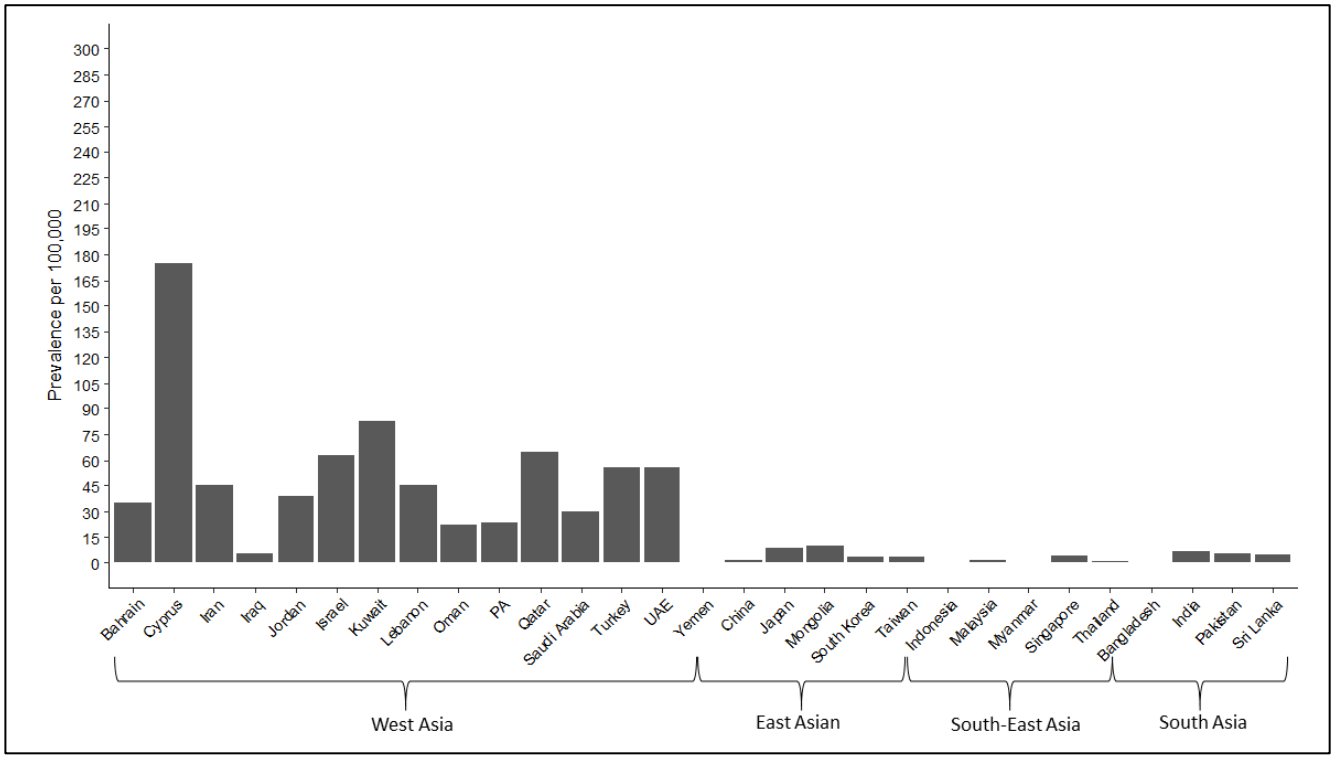
Nieto, 2012). For relatively rare, chronic conditions like MS, prevalence is a better measure of disease frequency than incidence, which seeks only to determine new cases within a specified period.

Prevalence is used as an indication of disease burden in different locations. However, comparisons of prevalence in different studies can be problematic for several reasons. Firstly, as touched upon above, diagnostic criteria may not be similarly applicable in all areas or countries, or different criteria may be used. Secondly, the reporting of MS may not be uniform in all countries, with some countries appearing to have a low prevalence as cases have not been recognised, access to clinical expertise has been limited, or it has simply not been reported consistently to researchers, while other countries appear to have a high prevalence owing to more advanced diagnostic techniques, more, and more experienced clinicians in the field, and thereby greater recognition. Those countries with improving diagnostic techniques may appear to have a rapidly increasing prevalence, as increased incidence will contribute to greater prevalence until all existing cases have been identified and only new cases are being diagnosed. Thirdly, MS prevalence may be disproportionately affected in different countries by survival: the longer the survival following diagnosis – and indeed therefore the earlier the diagnosis – the higher is the disease prevalence. Care therefore needs to be taken in interpreting prevalence from different regions.

In 2008 the Multiple Sclerosis International Federation (MSIF) produced a world atlas of MS epidemiology which was updated in 2013 (Multiple Sclerosis International Federation, 2013). This atlas collated worldwide information regarding MS and, although there are limitations regarding the data as outlined above, and that some countries, often lower income countries, did not respond, this is probably the most complete current picture of MS worldwide. Figures 2.2-2.6 show the prevalence of

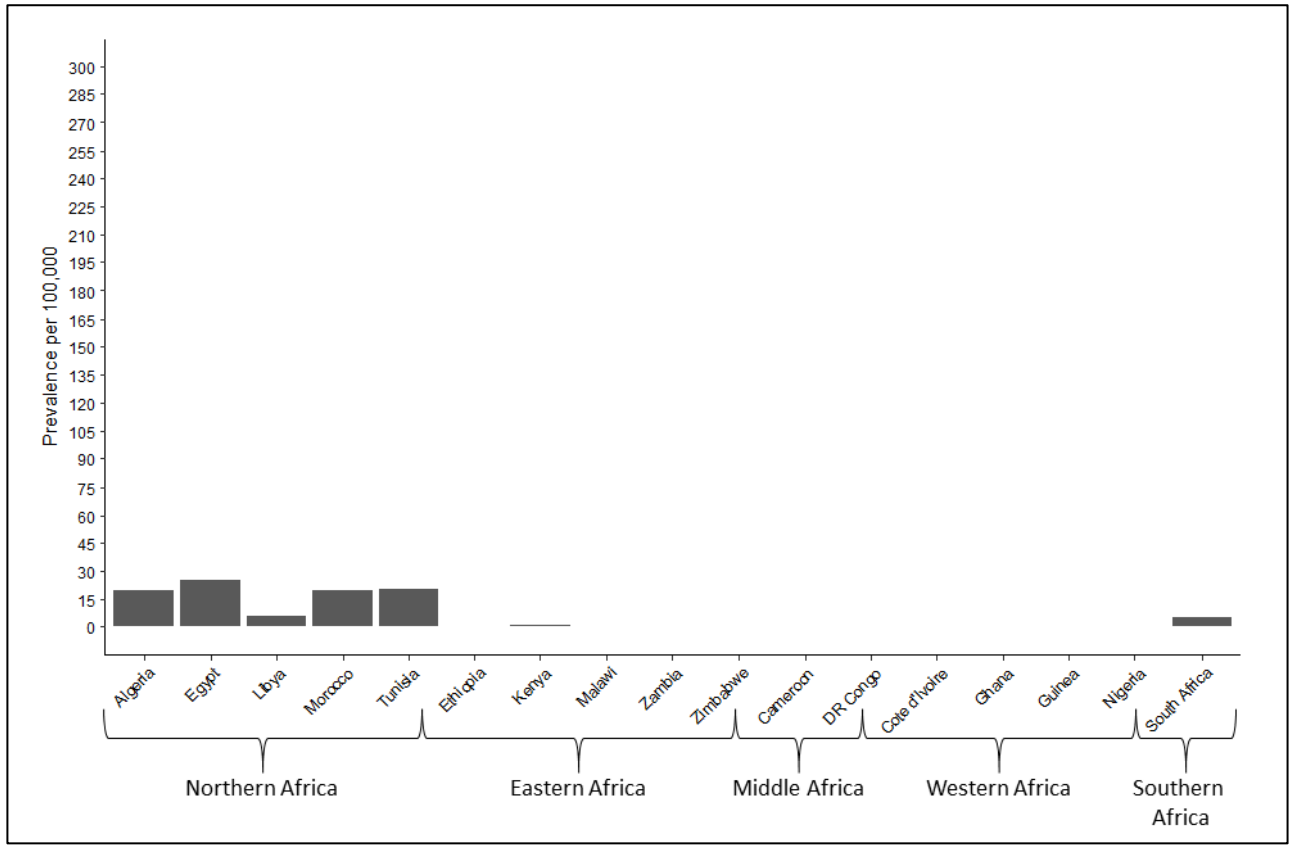
MS recorded in each country. All graphs show countries grouped alphabetically in UN-defined regions. European countries have been presented in Eastern and Western European groups as per the UN, however to highlight MS prevalence in the north and south, we drew an arbitrary line across Europe to further subdivide Eastern and Western Europe into countries situated in more northerly or southerly areas. Those countries that were approached for data by the by MSIF but did not contribute are also presented.

Figure 2.2 MS Prevalence in Asia



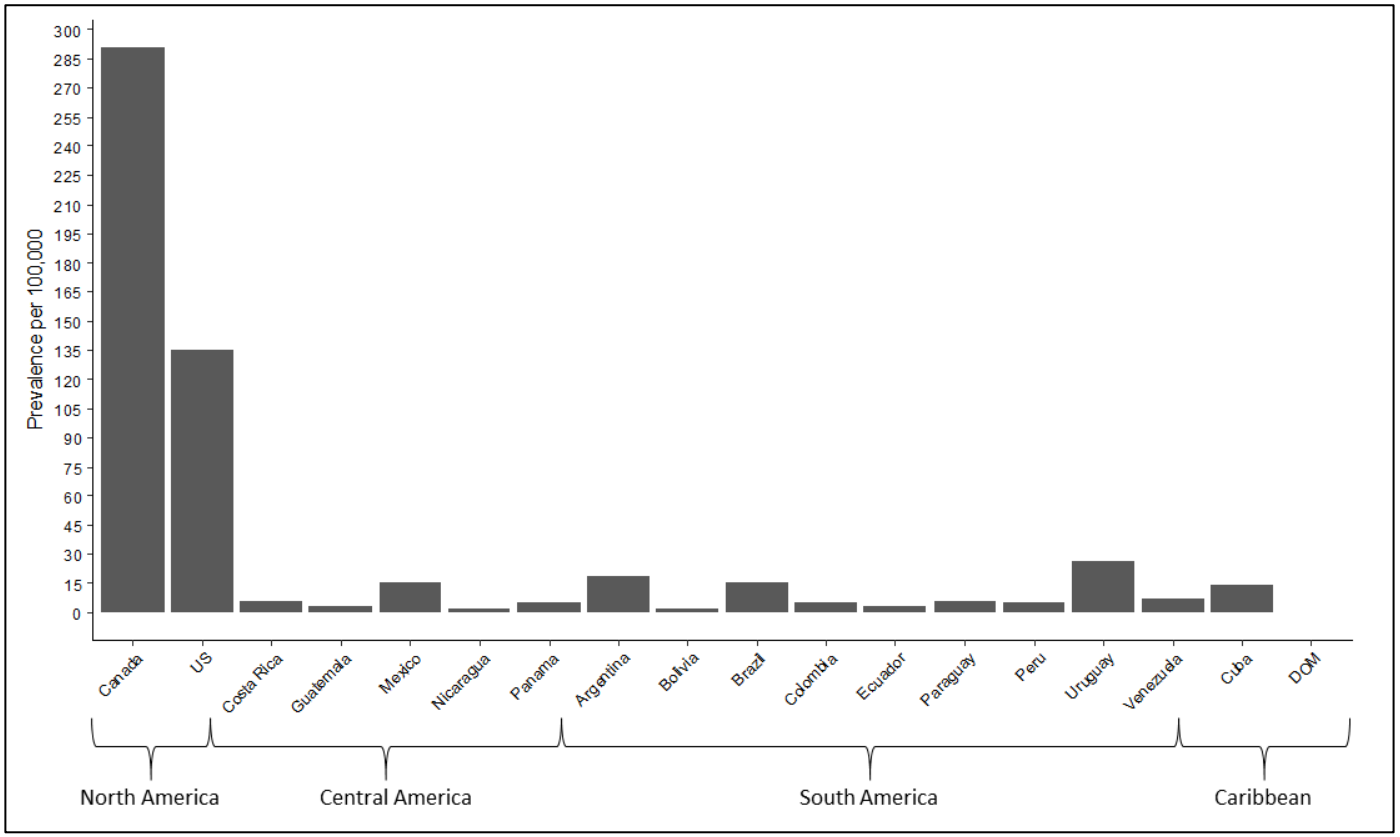

Abbreviations: PA: Palestinian Authority; UAE: United Arab Emirates

Figure 2.3 MS prevalence in Africa



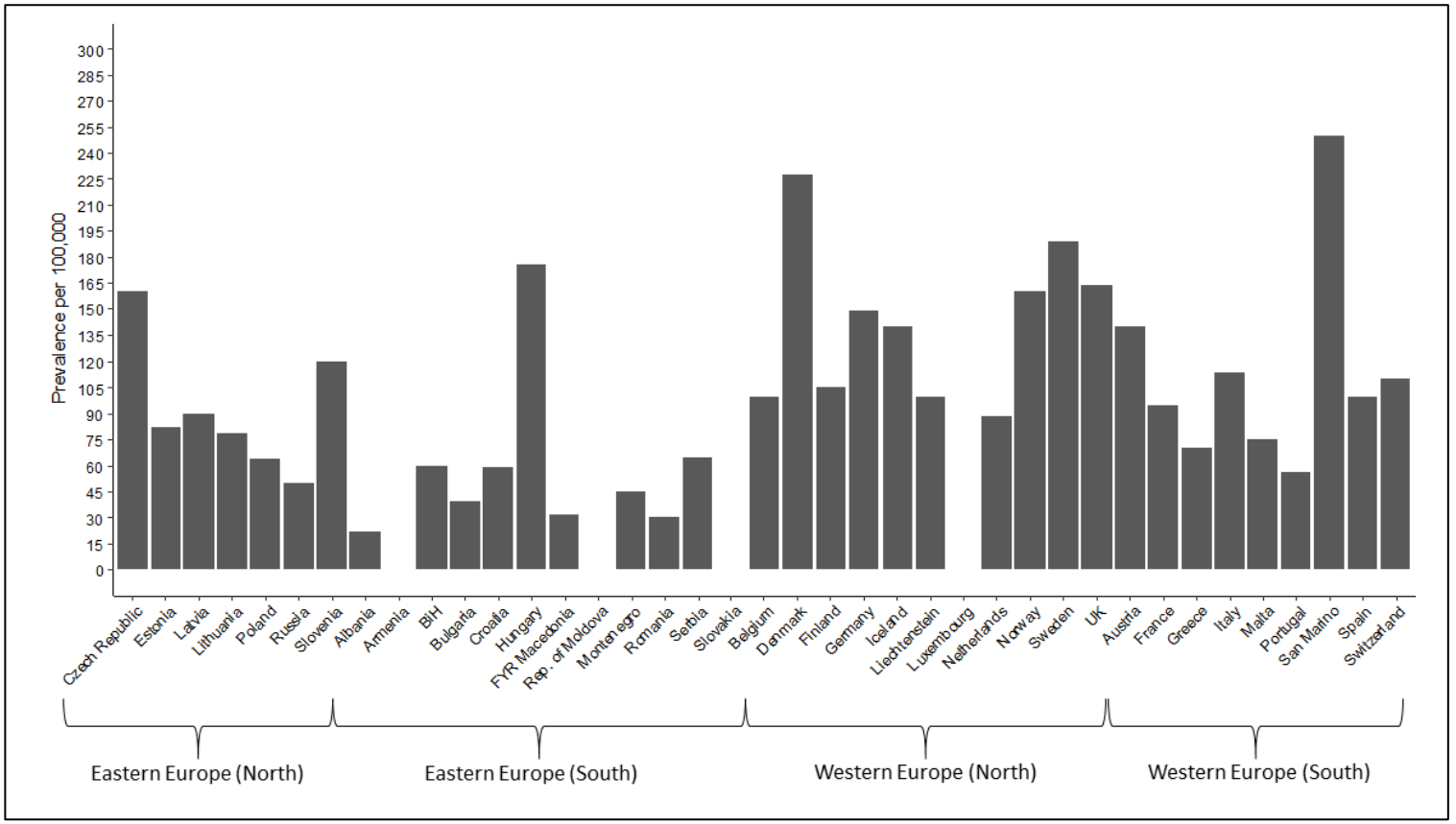

Abbreviations: DR Congo: Democratic Republic of the Congo

Figure 2.4 MS prevalence in The Americas



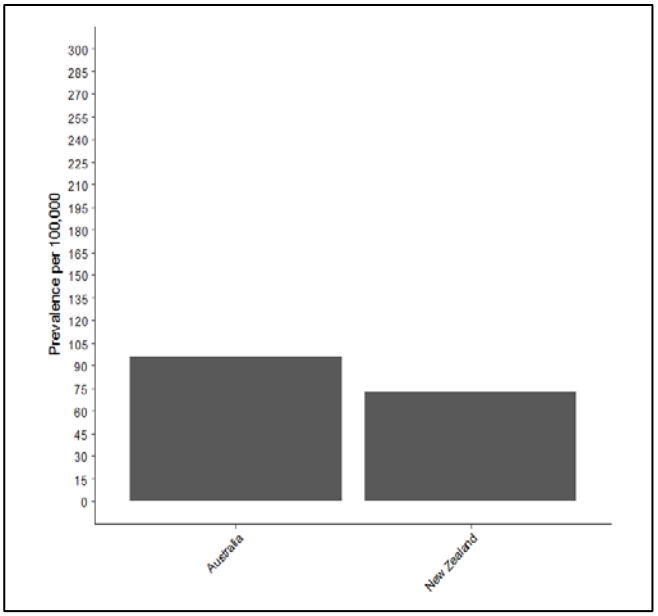

Abbreviations: DOM: Dominican Republic; US: United States

Figure 2.5 MS prevalence in Europe




Abbreviations: BiH: Bosnia and Herzegovina; FYR Macedonia: Former Yugoslav Republic of Macedonia; UK: United Kingdom

Figure 2.6 MS prevalence in Oceania




Overall, it is estimated that around 2,500,000 people worldwide live with MS (Compston and Coles, 2002). Despite such potential difficulties in comparing studies across different regions, a clear picture of MS distribution has been noted with more cases appearing in countries situated further from the equator. An argument against this latitudinal gradient of MS is that a number of prevalence studies have been inappropriately compared; small numbers of cases, ascertainment errors, selection bias in non-population-based studies, different methods for adjusting for age and sex alongside different diagnostic criteria can all introduce bias which complicate the comparison of such studies (Poser, 1992; Koch-Henriksen and Sørensen, 2010). Furthermore, the latitudinal gradient appears to be attenuated after 1980 when incidence is used as the measure rather than prevalence (Alonso and Hernán, 2008). However, a comprehensive review of 321 peer-reviewed studies and resultant meta-analysis found a significant positive correlation between latitude and MS prevalence in both northern and southern hemispheres, with two exceptions (Simpson S. Jr. et al., 2011). These exceptions include the Italian region, and the Scandinavia and North

Atlantic region, which both showed inverse latitudinal gradients. However, in the Italian region, adjustment for *HLA-DRB1* haplotypes (a strong MS risk factor) resulted in a positive gradient, suggesting that the distribution of *HLA-DRB1* haplotypes are responsible for the unusual latitudinal pattern. Although genetic data were not available for the North Atlantic and Scandinavia region, none of the prevalence studies that comprised the meta-analysis included large proportions of low-risk groups, such as the Sámi (indigenous people of northern Scandinavia). However, the low prevalence at these high latitudes may be partially attributable to Sámi genes. The authors also hypothesise that the dietary intake of vitamin D which exceeds other areas of Europe could be responsible for conferring a protective effect against MS, and thereby reversing the normal latitudinal pattern. The two differences that were noted could therefore be explained by specific genetic and/or behavioural-cultural factors within these regions (Simpson S. Jr. et al., 2011). The latitudinal gradient is discussed in further detail in Chapter 6.

MS affects more women than men; a number of studies originally identified a ratio of about 2:1 (Confavreux and Compston, 2006). However, several recent studies have found an increased incidence of MS in women over the past decades, leading to a greater prevalence of disease and increased female to male sex ratio, of between 2.3 and 3.5:1 (Ahlgren et al., 2011; Alonso and Hernán, 2008; Ramagopalan et al., 2010a; Wallin et al., 2012). A disproportionate number of women with MS also appears to be apparent in Orkney and Shetland and has appeared to increase in recent decades, with a collective female:male ratio of 1.3:1 in the 1970s to 2.14:1 in the 2000s (unpublished). Reasons for this increase in women and not men are currently unclear, however it appears that the difference may be arising from an increase in female RRMS cases, the phenotype of which may be more dependent upon environmental factors, while PPMS does not appear to change temporally (Ramagopalan et al., 2010a).

Furthermore, some studies have reported a latitudinal association with a temporal increase in female to male sex ratio to be greater in northern Europe than southern (Trojano et al., 2012), whilst others have found no such increase (Bostrom et al., 2013; Mackenzie et al., 2014). The sex ratio does appear to alter depending upon age at onset and disease course. A greater number of females are diagnosed with paediatric MS (Patel et al., 2009) and RRMS (Ramagopalan et al., 2010a), whilst the female preponderance tends to reduce in PPMS (Tremlett and Devonshire, 2006) with some studies reporting a higher frequency of PPMS in males (Polliack et al., 2001). Males also tend to have a faster progression of disability (Confavreux et al., 2003), which may only be present in relapsing MS (Ribbons et al., 2015).

The UK has an estimated MS prevalence of 203/100,000, with a female to male ratio of 2.4:1 which showed no sign of temporal variation between 1990 and 2010 (Mackenzie et al., 2014). Within the UK, Scotland has the highest prevalence (255/100,000) (Mackenzie et al., 2014). Orkney was identified in 2012 as having the highest MS prevalence within Scotland (402/100,000 – and indeed the world – with Shetland also very high (295/100,000) (Visser et al., 2012). The MS prevalence of Orkney and Shetland will be discussed in more detail in Chapter 4.

## 2.4 Summary

In this chapter, I have discussed how MS was recognised as a disease after a long history of anecdotal reports of temporary paralysis with increasingly debilitating symptoms. From here, I have shown how our understanding of the biological mechanisms and physical changes that occur in MS has evolved with extensive research, however the precipitating factor that causes the initial inflammatory response is still unclear. Despite this enduring uncertainty, studies have identified numerous genetic risks of which *HLA-DRB* alleles are most prominent, two environmental risk factors which have been consistently shown to increase risk,

namely smoking and EBV, and two slightly more controversial and interconnected risk factors, vitamin D deficiency, and inadequate UV exposure, which will be explored in detail later in this thesis. What is clear from the many prevalence studies of MS and reviews is that MS is widespread globally but tends to appear more frequently with increased distance from the equator: the origin of the UV and vitamin D argument.

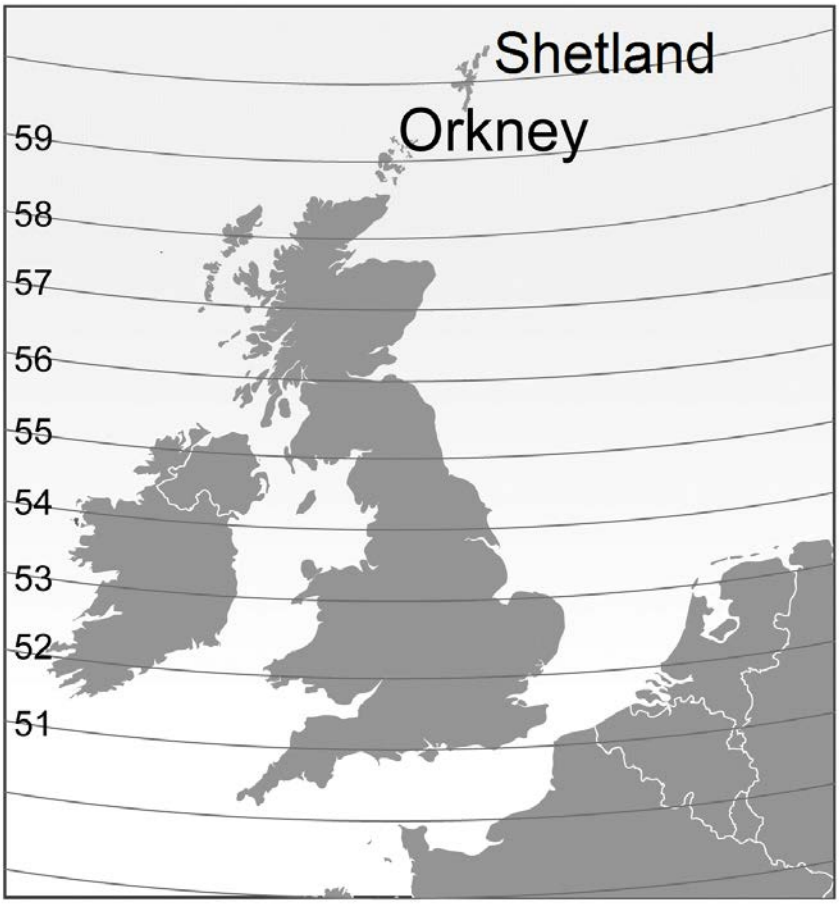
# CHAPTER 3. THE RESEARCH SETTING: ORKNEY AND SHETLAND

In this chapter, I introduce the islands of Orkney and Shetland, in which the primary research for this thesis was undertaken. Both the geographical location and relative isolation of the islands are important features to the studies of MS risk factors presented within this thesis. I begin this chapter by introducing the geographical location of Orkney and Shetland, and an overview of the general yearly climate. The geographical location and the local environment may have considerable implications for the population for two MS risk factors: vitamin D deficiency and reduced UV exposure, which I explore in detail in Chapters 7 and 8. I then go on to describe how the geographical location of the islands, alongside events over the centuries and in more recent years, have impacted the population structure and genetic makeup of the islanders today, resulting in distinct genetic isolates. Such genetic isolation may also have implications for MS. This is because in the absence of gene flow from the general population, rare and deleterious mutations can increase in frequency, and this can increase risk of disease in the population. I explore the possibility that the high prevalence of MS in Orkney and Shetland may result from an increased genetic risk in Chapter 5.

## 3.1 The Archipelagos

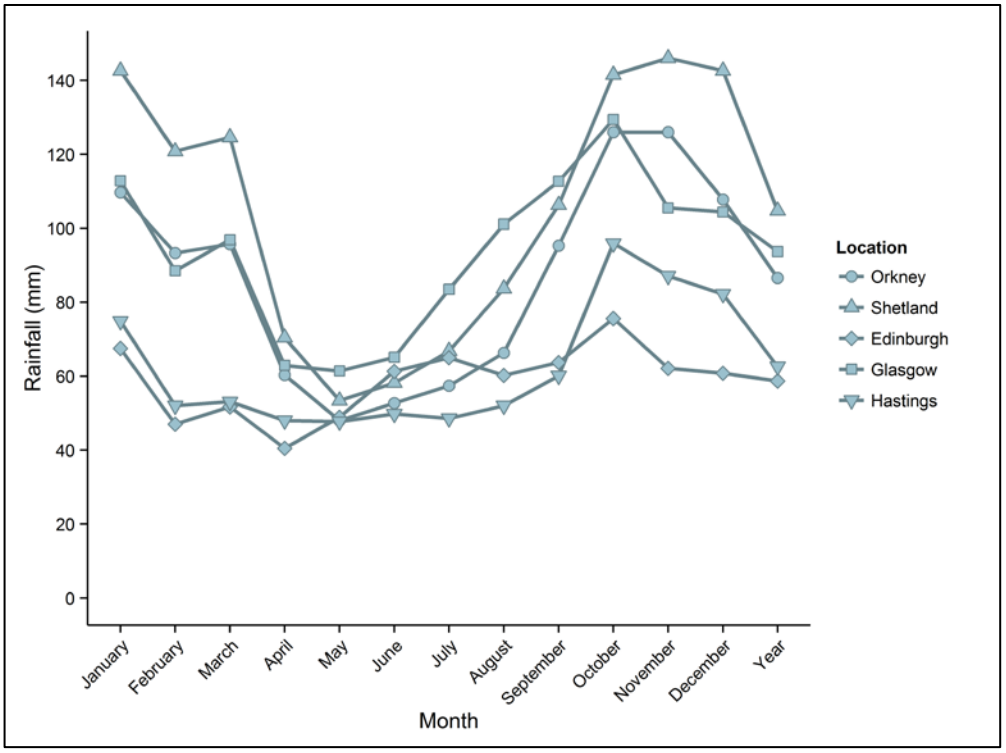
Collectively known as the Northern Isles, Orkney and Shetland are two distinct archipelagos forming the most northerly and remote areas of the British Isles (Figure 3.1). Orkney lies ten to sixty miles from the north coast of Scotland, across the Pentland Firth; Shetland is situated 50 miles north of Orkney, 170 miles southeast of the Faroe Islands and 225 miles east of Norway. The archipelagos sit between latitudes 58.8° and 60.7° north (similar to St Petersburg and Anchorage, Alaska), with the Atlantic Ocean to the west and the North Sea to the east. Orkney comprises about 70 islands, of which 17 are inhabited today; Shetland consists of over 100 islands of which 15 are inhabited.

Figure 3.1 Map of Orkney and Shetland with 51st to 59th lines of latitude



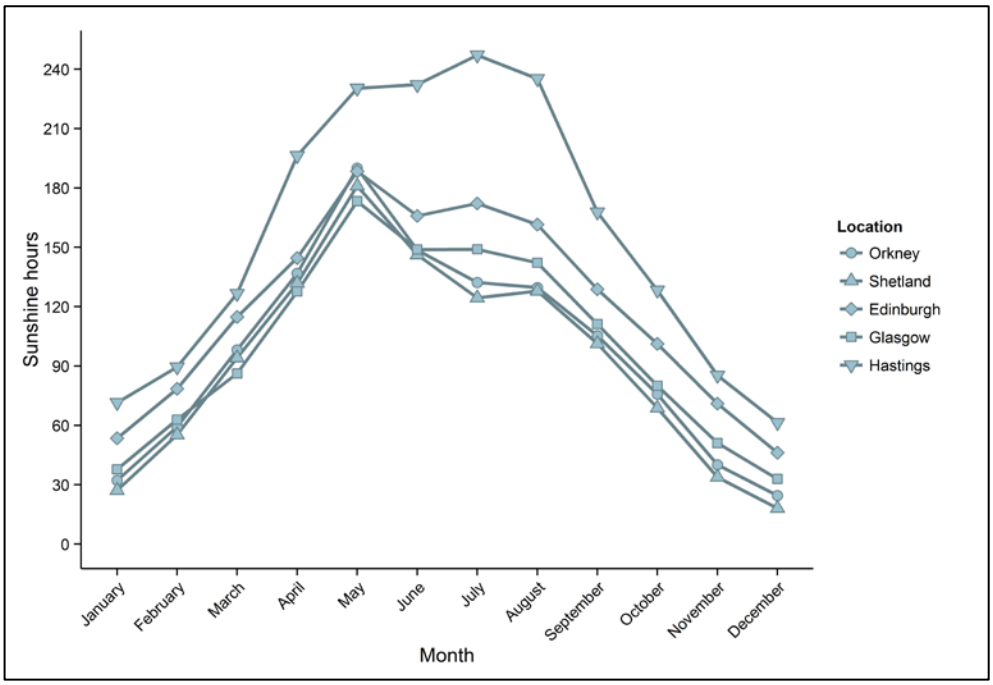

Orkney and Shetland are characterised by relatively high rainfall, few sunshine hours, and a temperate climate which is warmer than may be expected at such high latitudes, owing largely to the influence of the North Atlantic Current (Scottish Government, 2011) (Figures 3.2-3.4). To put the Orkney and Shetland climate into the context of the British Isles, Edinburgh and Glasgow, which are located further south in Scotland, and Hastings, located in the far south-east corner of England and which is noted for its warm and temperate climate, are included in the following figures. These other locations illustrate the diverse weather conditions within the UK and highlight that the climate in Orkney and Shetland is generally harsher and less temperate than the rest of the UK, with Shetland being particularly extreme.

Figure 3.2 Average rainfall, Orkney, Shetland, Edinburgh, Glasgow, Hastings



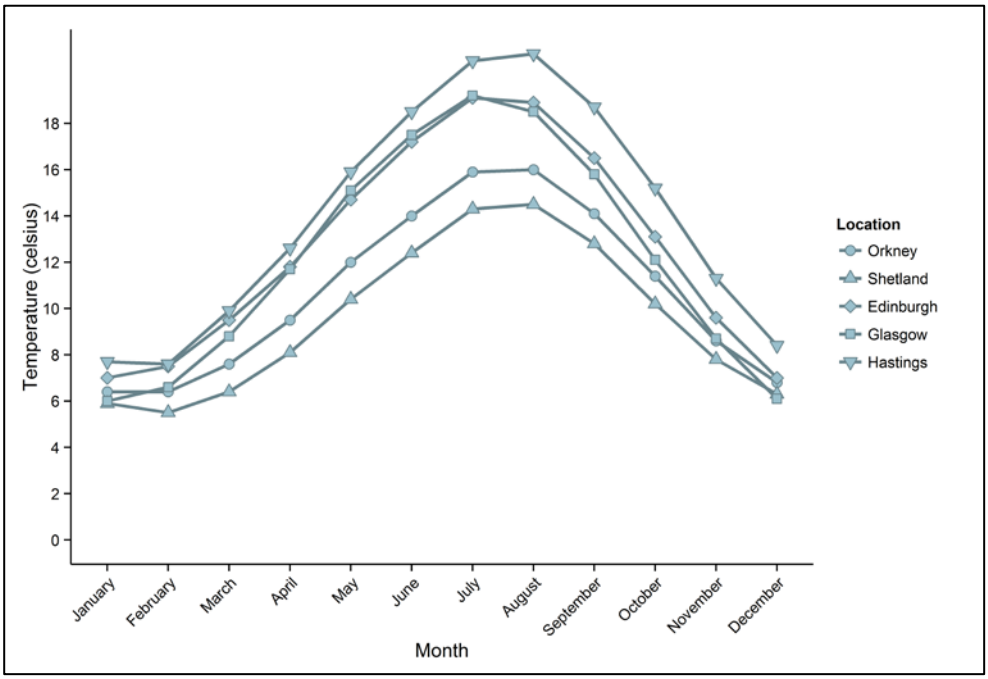

(Meterological, 2016)

Figure 3.3 Average sunshine hours, Orkney, Shetland, Edinburgh, Glasgow, Hastings




(Meterological, 2016)

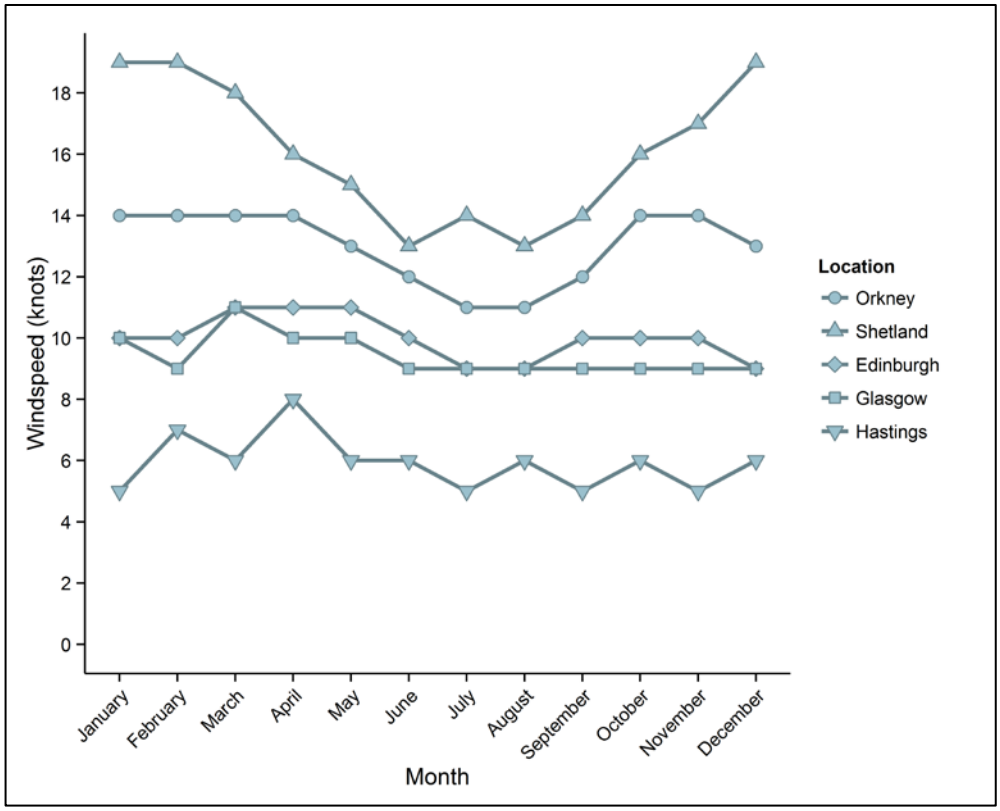
Figure 3.4 Average temperature, Orkney, Shetland, Edinburgh, Glasgow, Hastings




(Meterological, 2016)

As well as high rainfall, scarce sunshine, and a relatively warm climate for their latitude, the islands are characterised by high wind speeds (Figure 3.5). Such inclement weather, coupled with the remote geographical location, has meant that historically travel to and from the islands has been limited, leading to a relatively isolated population.

Figure 3.5 Average wind speed, Orkney, Shetland, Edinburgh, Glasgow, Hastings




(Windfinder, 2000)

Population isolates result from cultural or geographical isolation of a subpopulation for which there are genetic consequences. In the next section, I give an overview of when Orkney and Shetland were settled, and how the population has evolved into the current population of the islands today. I also discuss how modern industry, emigration, and immigration have shaped the current population structure and contributed to the Northern Isles gene pool.

## 3.2 Population history

### 3.2.1 The Mesolithic, Neolithic, Bronze Age and Roman era

Evidence for human habitation of Orkney and Shetland dates back to the Mesolithic era some 10,000 years ago. At this time, sea levels were lower than they are today and the land mass was much larger; what would have been the high ground to the earliest inhabitants are the islands that we see today (Saville and Wickham-Jones,

2012; Flemming, 2004). The Mesolithic people were nomadic hunter-gatherers; their itinerant lifestyle, alongside the changing landscape means that a very limited archaeological record remains. However, pieces of charred hazelnut (Archaeonews, 2007) and collections of worked flint (Towrie, 2007) provide evidence of their presence. The Neolithic period, beginning circa 4000 BC, is however well represented in Orkney and Shetland archaeology; the *Heart of Neolithic Orkney*, four monuments comprising two stone circles, a large settlement and a chambered tomb, is a UNESCO world heritage site paralleled in size and significance only by sites in Wessex (Unesco World Heritage Centre, 2016).

The Neolithic saw hunter-gatherers evolving into agricultural communities, which necessitated permanent settlement. The remains of houses, monuments, burial cairns, and the bones of domesticated animals give clues of their daily lives (Jones, 1999; Reilly, 2003), whilst the discovery of the Ness of Brodgar, a vast Neolithic complex of domestic and ritual buildings, suggests a focal point for people across Orkney (Towrie, 2009). Additionally two types of Neolithic pottery found in Orkney may denote different tribes, or may instead suggest the emergence of an elite group forming a stratified society (Towrie, 2015b; Brophy and Sheridan, 2012).

A number of finds in Shetland, including knives made of locally-mined felsite, and extensive field systems at the Scourd of Brouster (Mercer, 1987) show increasing human habitation and a growing agricultural tradition in Shetland in the Neolithic (Edwards and Whittington, 1998; Childe, 1938). Contact between Orkney and Shetland had been made by the end of the era, evidenced by their shared use of ceremonial maceheads (Brophy and Sheridan, 2012).

The Bronze Age appeared to touch Orkney only lightly; it is thought that this may have resulted from the change in climate as temperatures fell and rainfall

increased, making living and farming in Orkney difficult and travel to the islands more hazardous (Towrie, 1996). Complex archaeological settlements are however evident in Shetland (Childe, 1938; Guttmann, 2005). From 600 BC the inhabitants of both island groups began building roundhouses, the design of which became increasingly sophisticated over the next centuries. By 100 BC these roundhouses had become Brochs, large round stone defensive dwellings of which the remains of up to and over 100 are to be found in each of Orkney and Shetland. As it is estimated that each broch could house up to 50 people, the Iron Age population can be estimated to be up to 5000 people in both island groups (Boyce et al., 1973).

The Iron Age gave way to the Roman era; however, despite being fully aware of Orkney's existence (although maybe not of Shetland), the Romans themselves probably never set foot on the islands (Berry and Firth, 1986). The Iron Age in Orkney and Shetland is therefore often considered to stretch from 700 BC to AD 800 (Armit and Ginn, 2007). During the late Iron Age, it is thought that Pictish culture emerged amongst the indigenous Iron Age populations of Orkney and Shetland, and continued from about 300 to 800 AD. The name 'Pict' likely derives from 'Painted People', so-called by the Romans, however little else is known about this group of people. Their legacy on Orkney and Shetland largely comprises ornate stone carvings, sometimes with rune-like lettering (Berry and Firth, 1986).

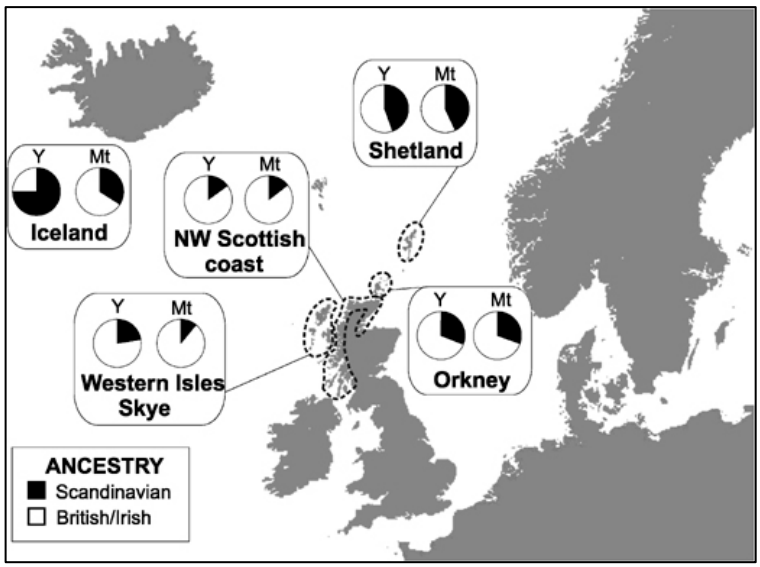
### 3.2.2 The Vikings, Earldoms, and annexation to the Scottish Crown

What is evident, however, is that from the 8th century AD Norsemen began to colonise Orkney and Shetland. Whether the Vikings integrated peacefully or effectively obliterated the preceding culture is a matter of great debate. To the present day, the Vikings most noticeably left their mark on the place names of Orkney and Shetland; the scarcity of pre-Norse place names suggests that the incomers may have overwhelmed the native population (Barnes, 2010; Lamb, 1993). However, archaeological arguments

for peaceful integration also exist; a Norse house found on the site of a Pictish settlement was found to contain Pictish artefacts suggesting integration of the two cultures (Ritchie, 1976).

As might be expected, genetic evidence in this North Atlantic region shows a large proportion of Scandinavian ancestry in Orkney and Shetland, although these data cannot tell us if invading Vikings took indigenous Pictish wives or if islanders of Scandinavian descent intermarried in later years with Pictish or Scottish immigrants. However, the invading forces of Orkney and Shetland appeared to settle as families. This is evidenced by roughly equal proportions of Scandinavian mitochondrial DNA, which is inherited from mothers, and Scandinavian Y-chromosomes, which are inherited from fathers, in Orkney and Shetland (Figure 3.6). Such equal proportions of maternal and paternal DNA strongly suggest that Scandinavian couples settled on the islands together. This finding is in contrast to those of Iceland and the Western Isles/Skye. Although it appears that whilst some couples settled in these locations together, the preponderance of Scandinavian Y chromosomes in comparison with Scandinavian mitochondrial DNA (particularly noticeable in Iceland) suggest that a large number of invading males took indigenous wives.

Figure 3.6 Map showing the proportions of Scandinavian and British/Irish mitochondrial DNA and Y-chromosome for admixed populations in the North Atlantic region




(Goodacre et al., 2005)

Politically turbulent times followed the Norse colonisation of Orkney and Shetland. The Norse Jarls, or Earls, who were based in Orkney, began by ruling Orkney, Shetland, parts of Caithness, and Sutherland (so-named for being south of Orkney), however in 1195 Shetland was removed from the Jarl's control as punishment for his involvement in a rebellion two years earlier (Imsen, 2010). Shetland became a Lordship and remained closely aligned with Scandinavia, however the Jarl's remaining area of responsibility focussed much more on lands around the Pentland Firth. Perhaps unsurprisingly connections with Scotland strengthened in Orkney in this time (Imsen, 2010).

The last Jarl was murdered in 1231 with no heirs apparent. The Jarldom then passed into the family of Angus, a Scottish dynasty who were also Earls of Caithness, however Orkney officially remained subject to Norway. The Earls of Angus continued to rule throughout the thirteenth century, eventually losing Sutherland to a different dynasty (Imsen, 2010). After the Angus Earls died out, the Strathearn and Sinclair Earls

respectively ruled Orkney, still under the Norwegian crown, and Caithness. However, in 1375 the Earldom of Caithness passed back to the Scottish crown leaving Orkney the only remaining part of the Earldom.

Norse rule remained until 1468 when Orkney was pledged as part of the dowry of Margaret, daughter of King Christian I of Demark, Norway and Sweden, who was betrothed to King James III of Scotland. The pledge was redeemable against a payment of 50,000 Rhenish Florins, however one year later, after no payment had been made, Shetland was pledged against a further 8,000 Rhenish Florins. Unable to redeem the islands, in 1472 Orkney and Shetland were annexed to the Scottish crown and absorbed into the Kingdom of Scotland (Miller, 1985).

Over the next several hundred years the Scottish population of the islands increased, although the extent to which new settlers arrived and contributed to the island populations is unknown. A large number of Scots were given land by contemporary Earls, and families with surnames such as Spence, Sutherland, and Sinclair, prominent in today's island populations, arrived during these centuries. A Shetland landowner noted in 1633 that the south parishes of Shetland "are, for the most part, Strangers from *Scotland*, and *Orkney* whose Language, Habite, Manners, and Dispositions, are almost the same with the Scottish…" (Monteith, 1845). The Court Book for Orkney and Shetland of 1614-1615 records the names Eriksone and Johnsdochter in Shetland; such local patronymics including Herculeson and Jarmson are still common in Shetland today. Orkney, conversely, had no patronymics recorded. Instead, the Orcadian names listed in the Court Book are typical Scots, such as Spence and Sinclair, or are instead derived from place-names, including Halcro, Clouston and Corrigall. Naming a farmer after his land is a Scottish tradition, suggesting that the Scots influence increased during these years in Orkney. However, these Orcadian place

name surnames, like the Shetlandic patronymic surnames, are highly enriched for Norse Y chromosomes, showing that whatever the origin of the tradition, Orcadian place-name surnames were overwhelmingly founded by Norsemen (Wilson et al., 2001). Y-chromosome analysis of an Orkney-wide sample suggests an approximately 53% Norse and 47% Scots split (Wilson, unpublished). Further evidence for the sizeable infiltration of Scottish people and culture comes from the loss of the Norn language, which was derived from Old Norse and had local dialects in each archipelago. By 1800, the language was no longer spoken with any proficiency (Barnes, 2010), suggesting that the Scots way of life had largely overtaken the old Norse ways.

### 3.2.3 The impact of industry, modernity, emigration and immigration

A well-documented history of Orkney's involvement with the Hudson's Bay Company (HBC) exists. Records kept by the HBC show that from 1702 the company started calling at Stromness in Orkney to recruit men for their labour force. Following the Act of Union between Scotland and England in 1707, these recruitment visits began on a yearly basis reaching a zenith between 1771 and 1799, when 80% of the 529 HBC employees were Orcadian. A large number of Orcadians settled in Canada and married Cree women, one of the First Nation peoples. They thereby bestowed local Orcadian names on the new lands, and surnames such as Flett, Foubister, and Linklater, on the native population (Troup, 2003; Moffat and Wilson, 2011). At least five instances are known of men returning to Orkney with Cree wives (Berry and Firth, 1986), thereby contributing Cree genes to the modern Orcadian genepool.

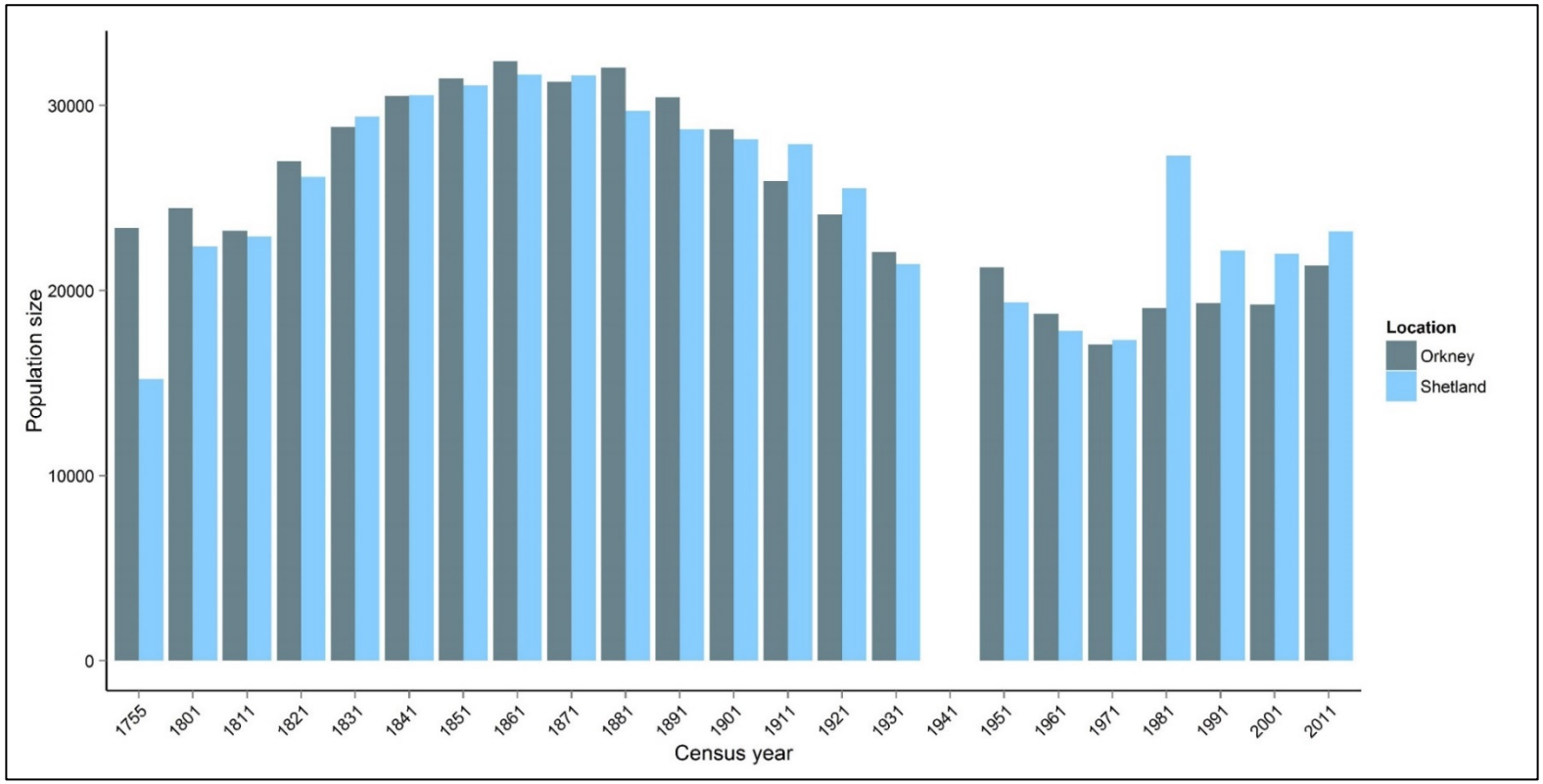
A regular influx of Dutch traders is also well documented. The Dutch became familiar with Shetland from the herring fishing that brought them to the lucrative waters from the mid-1600s. In 1633 Montieth wrote that "All the natives can speak the *Gothick* or *Norwegian* tongue… by reason of their Commerce with the *Hollander*, they promptly speak *Low-Dutch*" (Monteith, 1845). In 1700 a visiting churchman noted that

"those who commonly frequent this country and trade with the inhabitants are Hamburgers and sometimes Bremers and others… and in several places set up booths or shops where they sell liquors, as beer, brandy, etc., and wheaten bread… they also sell several sorts of creme-ware, as linen, muslin, etc" (Brand, 1701). The Dutch connection endured, despite the Anglo-Dutch wars that ran intermittently between 1652 and 1784. Sir Walter Scott noted in his diary of his 1814 vacation: "At Lerwick the Dutch fishers had again appeared at their old haunts. A very interesting meeting took place between them and the Lerwegians, most of them being old acquaintances… In general they are extremely quiet, and employ themselves in bartering their little merchandise of gin and gingerbread for Zetland hose and nightcaps" (Grierson, 1932). Despite such a connection, it is considered that the Dutch left no enduring cultural mark on the islands (Grydehøj, 2013).

Trade and industry were clearly bustling on the islands at this time with large numbers of people both coming and going. However, our knowledge of the Orkney and Shetland populations, and how they were affected by this movement, are limited, as few early attempts were made to systematically map the industry, economy, and population of Scotland. The first successful population census was produced in 1755, followed by the Old and New Statistical Accounts of Scotland, produced in 1799 and 1832 respectively. The Third Statistical Account was published in 1951. Enumerators provided statistical returns from 1801 to 1831, which detailed the population sizes of Scottish districts, and in some cases noted the details of householders and their occupations. However, the quantity of information collected was dependent upon each individual enumerator; additionally, survival of the records is poor (personal communication, Martin, 8 April 2016). Full censuses, listing the name and occupation of each person, were collected with increasing detail from 1841 onwards. However, all

these statistical returns provide a reasonable estimate of the population sizes of the islands, and show how the population sizes have fluctuated over the years (Figure 3.7).

Figure 3.7 Population sizes for Orkney and Shetland, 1801-2011




*Note: 1755 data from Webster Analysis of Population*

*No census was taken in 1941 due to the Second World War*

Between 1755 and 1801, the population in Shetland increased significantly, however Orkney's population remained quite stable. This constant population size has been attributed to significant emigration from Orkney which prevented population growth (Thomson, 2008). Slow population growth continued in Orkney, with a slight decline in Shetland, between 1801 and 1811. A large number of Orcadian and Shetlandic men were pressganged into, or volunteered for, the navy at this time, to fight in the French Revolutionary Wars (Miller, 1985). In 1884 it was estimated that at the preceding war, 12,000 men from Orkney and Shetland had been in the navy (Fea, 1884); a considerable number for the population size.

Both groups of islands grew in population from 1811, peaking in 1861. Throughout these years, the herring fishing industry boomed and continued to increase through to the early 20th century. Scottish boats followed the herring from the West Coast to Shetland, Orkney, Wick and as far south as Lowestoft (Berry and Firth, 1986). It was estimated that an additional 4000 people arrived on these boats in Orkney each twelve-week herring season before moving on, whilst 21,201 people were involved in the industry at its peak in Shetland (Gordon, 1845; Simpson, 2009). It is from this period that the saying came into being, that a Shetlander is a fisherman with a croft and an Orcadian is a farmer with a boat (Thomson, 2008), an adage that continues to summarise the islands today.

While Shetland's craggy terrain and hilly moorlands resulted in meagre subsistence crofting, the fertile land in Orkney lent itself to generations of farming. Farming practices in Orkney began to change with the Agricultural Revolution, which began in the 18th century and led to a change from subsistence farming to a market economy. Lairds recruited people, likely from mainland Scotland, skilled in new farming techniques and who had lost land in the Clearances (Berry and Firth, 1986).

Following the end of the First World War large taxes on big landowners led to a number of small farms being sold to their occupiers (Berry and Firth, 1986).

Communication also became easier during the 19th century. The first commercial enterprise offering ease of access and departure was a steamboat service, run by the North of Scotland and Orkney and Shetland Steam Navigation Company, which was founded in the 1830s to carry shipments of food and passengers (Special Collections Centre, 1810-2002). However, then and still today, sea travel is at the mercy of storm-force winds and rough conditions (Towrie, 2015a). Although some construction of roads had begun in Orkney from the 1760s, a programme of substantial road building began in 1857. Shetland, whose roads until this time had been minimal, saw systematic construction of roads between 1849 and 1852 (Moodie Heddle and Mainland, 1920).

These decades of population increase were followed by over a century of steady population decline. Despite periods of ample employment opportunity, many islanders were attracted by a new life in the colonies or America (Tudor, 1883). Enough people of Orcadian and Shetlandic descent had settled in America by the last quarter of the 19th century for the establishment of the *Orkney and Shetland American* newspaper. An 1889 survey of emigrants collected 3000 Northern Isles names, and the editor estimated that the same number again had not replied (Thomson, 2008).

During this period of emigration, more men were leaving Orkney and Shetland than women. This selective emigration led to greater numbers of women remaining in Orkney and Shetland. In 1861 Shetland had 143 females to every 100 males, while Orkney had 117 females to every 100 males (Census of Scotland, 1861). Although the home-knitting and herring industries, and other occupations, kept women gainfully employed, some saw the disparity as a burden:

> By reason of this constant drain of the male part of our Inhabitants, we must necessarily have a much greater number of women than men among us, who being destitute of any kind of employment, must ly as an useless burden on the country; and, what is still worse, many of them must likewise be destitute of husbands, by which means they degenerate into that wretched species of beings called Old maids, so that to all our other evils, that of being pestered with the female Grimalkins is likewise added (Fea, 1884).

The World Wars saw a large influx of military personnel, garrisoning the islands and manning the naval bases (Berry and Firth, 1986; Blackadder, 2007), while Orcadian and Shetlandic men were conscripted. In the Second World War Shetland upheld its Norwegian connections as civilians sailed to Nazi-occupied Norway in boats disguised as fishing vessels. There, they delivered weapons and supplies to the resistance, and rescued agents fearing capture by the occupying forces (Howarth, 2008). Orkney's wartime heritage lies in Scapa Flow – the naval base for the Grand Fleet in both wars – where shipwrecks of sunken vessels are still visible, and in the remains of gun batteries around the islands. The most significant change, however, is possibly the construction of the Churchill Barriers, a series of causeways linking the islands and further blocking off the eastern approaches. Constructed by Italian prisoners of war, these concrete barriers linked the previously separate isles of Lamb Holm, Glimps Holm, Burray and South Ronaldsay with the Mainland of Orkney. As well as permanently changing their topography, these barriers also provided easier movement throughout the south isles and to the mainland.

Charting the population change by registration district in Orkney from 1891, near the beginning of the decline, to 1961 (Figure 3.8), clearly shows a greater loss from the island communities (coloured green), compared to mainland Orkney (coloured blue). The only parish to increase in population was the capital, Kirkwall. Slightly smaller losses were seen in places with small towns or large villages, for example Stromness, Harray and Sandwick.

Although less clear because of the registration district boundaries in Shetland which group some island and mainland parishes together (coloured brown), it is still evident that the capital of Lerwick on the mainland (mainland districts coloured blue) was the only parish to increase in size (Figure 3.9). The island parishes, (in green), also incurred losses. Again, the parish with the next largest settlement shows the smallest decline: the village of Scalloway is in the Tingwall, Whiteness & Wiesdale parish.

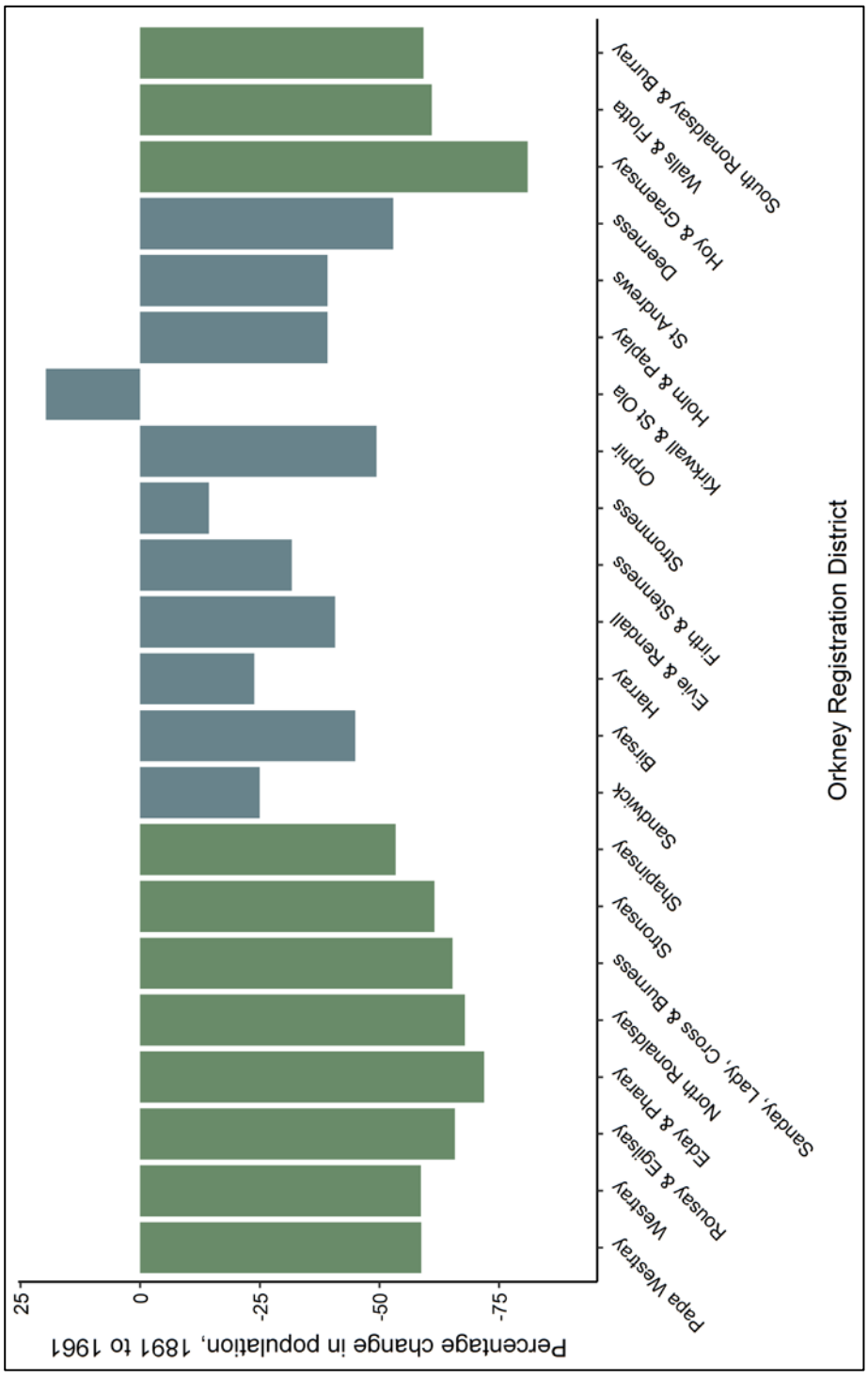
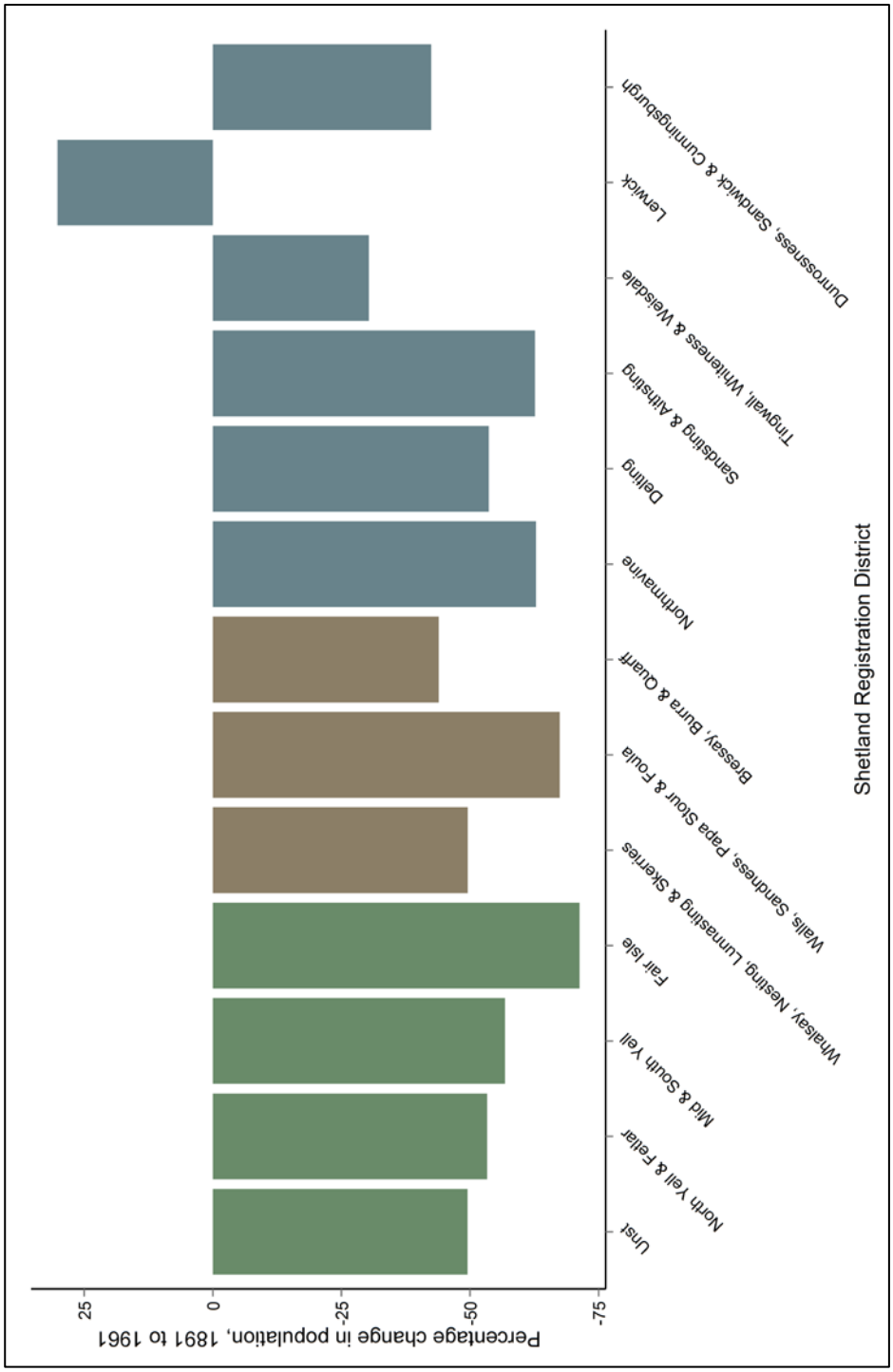



Figure 3.8 Orkney percentage change in population be registration district, 1891-1961

Figure 3.9 Shetland percentage change in population by registration district, 1891-1961



The 1970s and 1980s saw a substantial increase in the population sizes of Orkney and Shetland as it became increasingly easy to travel. Loganair started a chartered air service to Orkney in 1967, and to Shetland in 1970 (Loganair, 2010), which, until recently would be regularly grounded by thick sea mist (Towrie, 2015a). The discovery of North Sea oil and the concomitant industry that built up brought in a number of workers and their families. Additionally, urban refugees, seeking a change from town and city life, were also responsible for the increasing population (Forsythe, 1980). Through this period, considerable immigration from English cities unfolded. The population of Shetland swelled by almost 10,000 between 1971 and 1981, an increase of 36.5%; Orkney's population increased by the more modest but still considerable 10% over the same period. In 1986, Egilsay had one native-born Orcadian among the remaining population of 24; further, an unnamed island with a population of 186 in 1981 comprised 41% incomers (Berry and Firth, 1986).

Since 1991, the population sizes in Orkney and Shetland have remained more stable, although they continue their upward trajectory possibly because of a longer-lived and aging population. At the 2011 census, the Orkney population totalled 21,349, a near-11% increase on the previous census. Compared to mainland Scotland, Orkney has an older age profile, and an increasing elderly population. By gender, 49.5% of the population were male in 2011 (HIE, 2014a), compared with 48.5% on the Scottish mainland. The 2011 census also revealed that relative to Scotland, Orkney had a higher share of employment in agriculture, forestry or fishing (10% of the workforce compared to 2%), in construction (11% compared to 8%), and in transport and storage (9% compared to 7%). The workforce in Orkney and mainland Scotland were roughly equal in the mining, quarrying and utilities industry (~3%), accommodation and food services (~6%), education (~8%) and health and social work (~15%). Skilled trades

were also more numerous in Orkney compared to Scotland (22.5% compared to 12.5%) (HIE, 2014a).

Shetland, meanwhile, had a total population of 23,167 at the 2011 census, which was an increase of 5.4% on the previous decade. However, compared to the rest of Scotland Shetland has a younger age profile. Although there has been an increase in the older population in Shetland, proportionately it is a smaller increase than seen elsewhere. By gender, Shetland has a greater proportion of males than elsewhere in Scotland at 50.8% (HIE, 2014b). The 2011 census showed that relative to mainland Scotland, and like Orkney, Shetland has a higher proportion of the workforce employed in agriculture, forestry and fishing (~6%), construction (11%), and transport and storage (10%); and also education (~10% compared to ~8%) and health (16% compared to 15%). Similar to Orkney, skilled trades are more common (20% of the workforce), as are process, plant and machine operatives (10% compared to ~7%) (HIE, 2014b).

## 3.3 Population structure of Orkney and Shetland

A number of events over the centuries and more recently have therefore shaped the origins of today's Orcadians and Shetlanders. However, despite the fluctuating population size, emigration, and immigration over the years, Shetland and Orkney are genetic isolates.

### 3.3.1 Orkney and Shetland's DNA

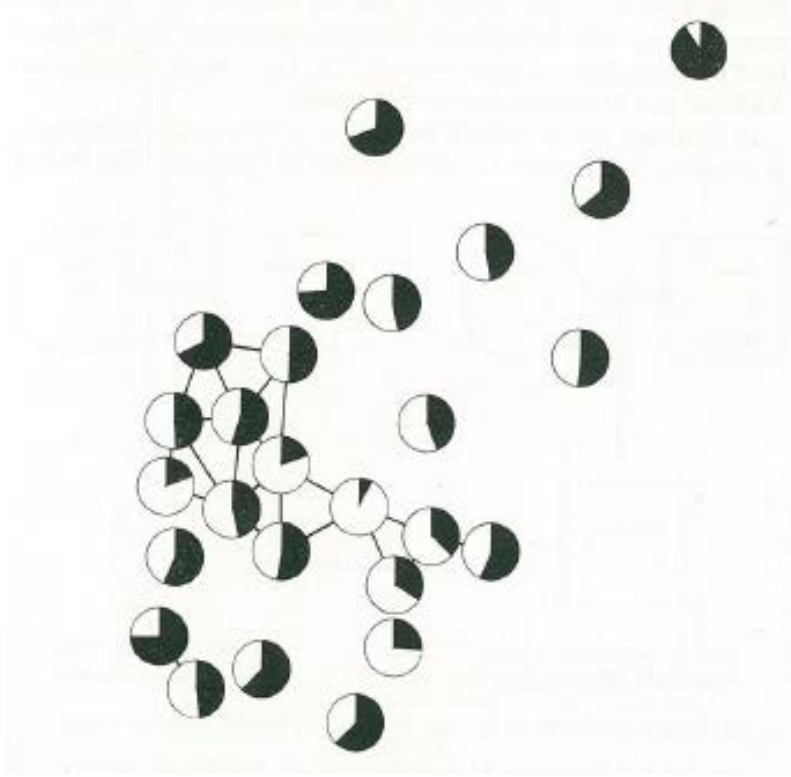
The genetic structure in Orkney has been more frequently studied than the genetic structure in Shetland, and is therefore understood in greater detail. A 2015 study (which excluded Shetland) found that Orkney was the most genetically distinct compared to the other UK populations studied (Leslie et al., 2015). Furthermore, within Orkney, people of Westray, Stronsay and Sanday ancestry were found to be identifiable from DNA alone (O'Dushlaine et al., 2010; Leslie et al., 2015). This demonstrates that within Orkney, at least three islands have maintained considerable isolation (O'Dushlaine et al., 2010), and it is likely that other outlying districts would show a similar pattern. The picture within Shetland is considerably less explored and therefore more ambiguous. However, a 2005 study found that Shetland's DNA is distinct from Orkney, and is possibly the most genetically differentiated of the ten Scottish populations studied (Vitart et al., 2005). This uniqueness of and within Orkney, and of Shetland, may have implications for genetic risks in this population. A recent study reported the identification of a highly prevalent *BRCA1* mutation in a region in the Tyrol, in 238 apparently unrelated families, which correlates with an increased incidence of breast and ovarian cancers (Pölsler et al., 2016). There is a possibility that a similar, as-yet unidentified, genetic risk for MS could be found in our study region, or that small differences in the frequency of common genetic variants might act collectively to increase the risk.

### 3.3.2 Inter-relationships

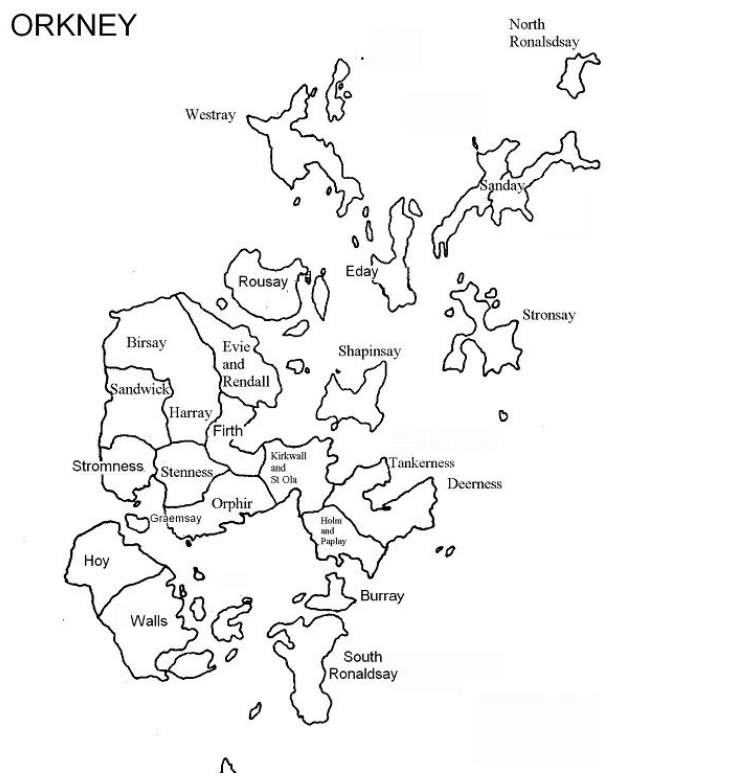
As well as information about the origins of the islanders, genetic differences and similarities among the various communities that make up Orkney and Shetland can be deduced from information about the inter-relationships of the island inhabitants (Roberts et al., 1973). Particularly where population sizes are small, recent increased movement between communities can have a large impact on genetic variation (Roberts et al., 1973). Demographic statistics available from the 1841 census onwards help to illuminate how the population structure may have changed over the last one to two hundred years (Harrison and Boyce, 1972).

One method of assessing the degree of isolation of a community is by examining the proportion of endogamous marriages (Figure 3.10), defined as a marriage in which both partners are from the same community. In 1861, there were already differences among the island parishes in the degree of isolation. Kirkwall and Stromness, the two most populous parishes of Orkney (containing small towns), showed the least endogamy while the North Isles, including Westray, North Ronaldsay and Sanday, had the highest degree of isolation by endogamous marriage (Roberts et al., 1973). Some of the South Isles and outlying Mainland parishes such as Birsay and Deerness also show considerable parish endogamy.

Figure 3.10 (Left) Proportions (shaded areas of circles) in 1861 of marriages in which both partners were born in the same parish



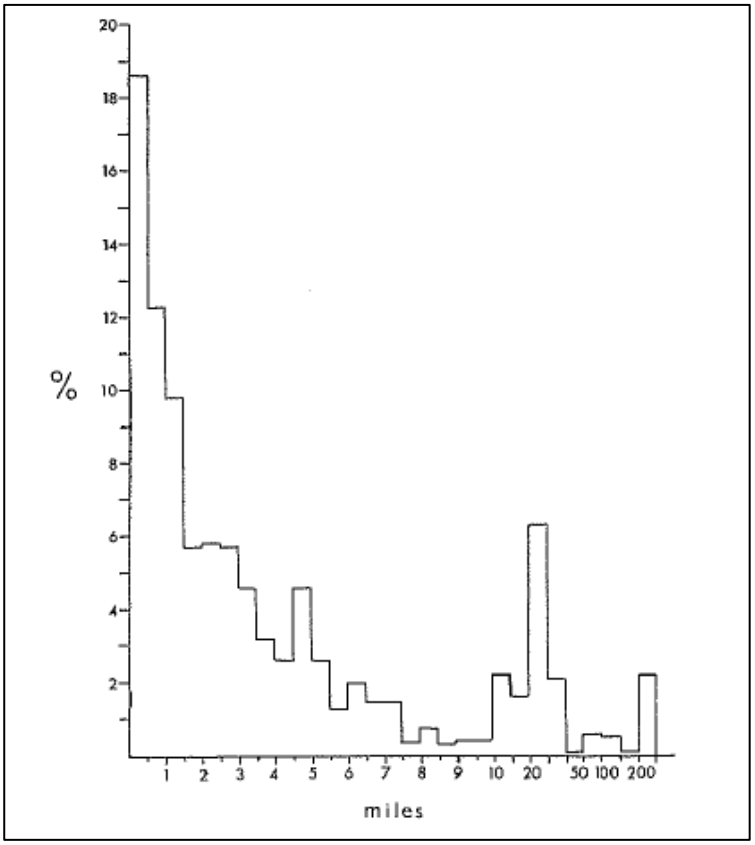
(Roberts et al., 1973)




(Hnolt, 2016)

Analysis of marriage certificates between 1855 and 1965 on Sanday, which had a proportion of roughly two-thirds endogamous marriages in 1861, found that the distances between the residences of husbands and wives at the time of marrying in Sanday was largely negatively skewed (Figure 3.11). Few couples comprised one partner born outside of Sanday. Thirty percent of marriages were between spouses living 1 mile apart or less, with a further 28% between those living from 1 to 3 miles apart (Roberts et al., 1973). A similar pattern was noted on Westray, where the distance between the residences of future spouses was up to one mile for over 40% of couples marrying between 1855 and 1974 (Collacott, 1979).

Figure 3.11 Distribution of distances between residences at time of marriage of husbands and wives marrying on Sanday between 1855 and 1965



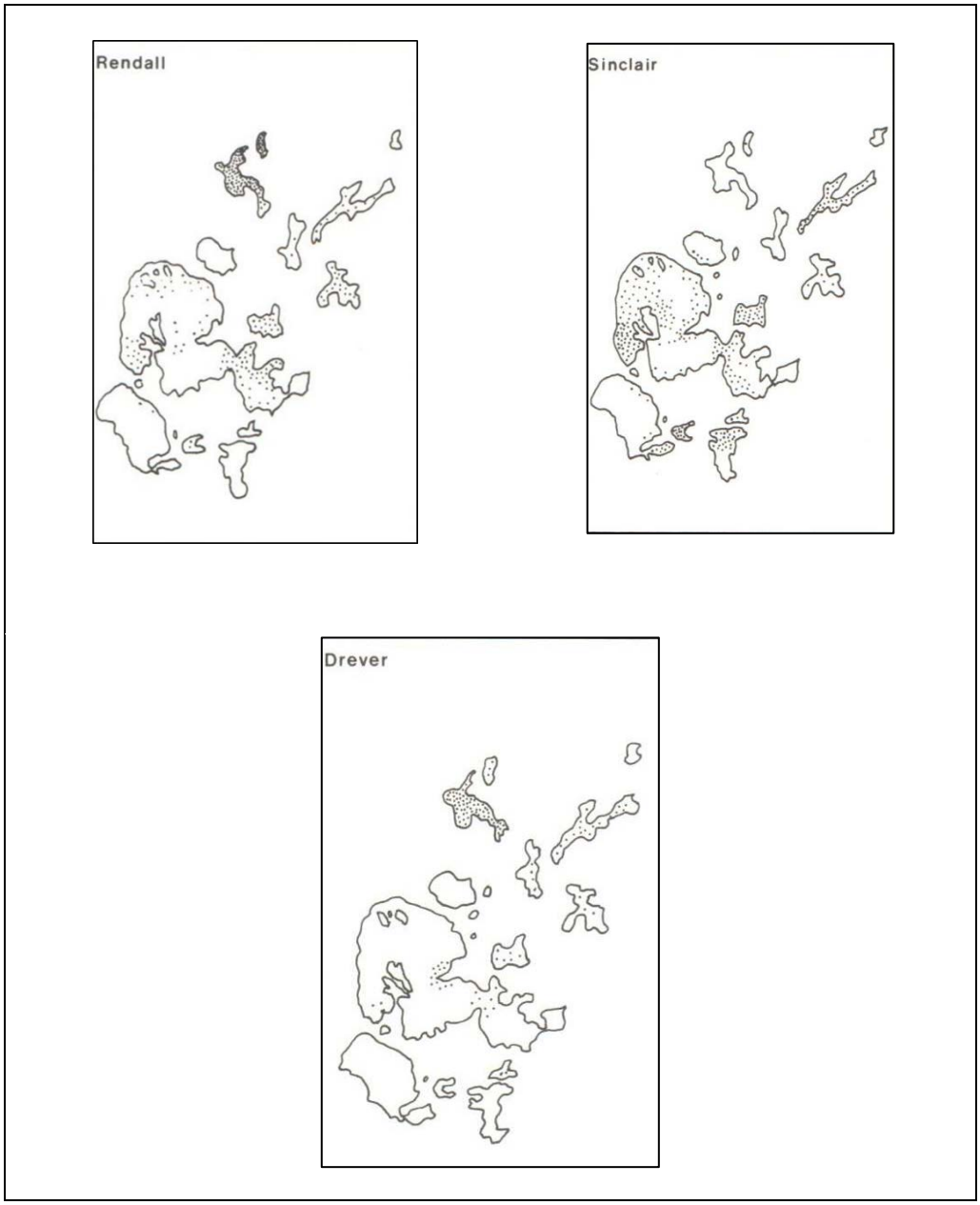

(Roberts et al., 1973)

Whilst Figure 3.11 illustrates that spouses mainly lived close to each other at the time of marriage with just a few exceptions, it does however fail to capture who had

been living in the isle all their life, and who may be a newcomer. The end of the Sanday study preceded the large influx of people from the 1970s, and it may therefore be fair to assume that there were few newcomers. Meanwhile, the proportion of Westray residents marrying from within Westray had barely changed from 1855 to 1974 (Collacott, 1979). However, from the 1970s onwards, it is more likely, due to the increased emigration, that more couples may comprise one partner who had been born outside the isles or even outside Scotland.

A further method of illustrating the population genetic structuring present in the islands is by surname analysis. Data from the Orkney phone book from 1979 to 1980, after the period of mass immigration to the islands, give an idea of how such immigration may have altered the population structure (Lamb, 1980). Figure 3.12 highlights three surnames: Rendall, Sinclair, and Drever, each of which is particularly prominent in different parishes. Rendall shows an enrichment in Westray, but is also seen in Stronsay, Shapinsay, Stromness, and Kirkwall. Sinclair is a bit more widespread but nonetheless is concentrated in Sanday, Shapinsay, Stromness, Flotta, South Walls, and in the northern parish of South Ronaldsay. Drever likewise clusters in Westray with some scattering over the other North Isles and mainland. These examples demonstrate ongoing male lineages with little evidence of panmixia, meaning that there are at least still partially separate gene pools in different parts of Orkney.

Figure 3.12 Orkney surnames and parishes in which they cluster




(Lamb, 1980)

However, it is important to note that while this structuring remains within the isles, the modern movement of people into and out of each parish are different and may have disproportionately affected some parishes. Each parish and island community has its own unique history against which the remaining population today is shaped. The proportion of remaining indigenous populations are therefore quite different in each location. Agricultural parishes may have been more likely to retain their population to

work the land; fishing communities more likely to lose their population as they either followed the fish, or, subsequent to the collapse of the fishing industry, sought employment elsewhere.

## 3.4 Summary

In this chapter, I have introduced Orkney and Shetland and have discussed their relative isolation caused by both their geographical location and by the characteristic inclement weather, which has historically discouraged travel to and from the islands. Further, I have discussed the population history of Orkney and Shetland, from the earliest settlers through to the present day, and illustrated how the populations have been shaped by industry, emigration, and immigration. Finally, I have discussed the present day population structure of the islands, and have shown that although increased movement of people has affected areas differently, analyses have shown that overall Orkney and Shetland remain genetically distinct: a product of their isolation.

# CHAPTER 4. DISTRIBUTION OF MULTIPLE SCLEROSIS IN THE NORTHERN ISLES

## 4.1 Introduction

Evidence from past and recent studies suggest that Orkney and Shetland have a very high prevalence of MS. In 2012 prevalence in Orkney was estimated to be the highest in the world, with the prevalence in Shetland being only slightly lower (Visser et al., 2012) and possibly the third highest worldwide after Atlantic Canada (Beck et al., 2005). This finding followed a history of studies of the region, each observing a high MS prevalence in the Northern Isles compared to the rest of the UK and globally. Table 4.1 presents an overview of some high prevalence regions within high prevalence countries, as well as the old and new prevalence studies undertaken in Orkney and Shetland.

Table 4.1 Selection[1] of prevalence studies of Orkney and Shetland, the UK and globally

| Authors | Year of Pub. | Diagnostic criteria | Region | Prevalence/ 100,000 (95% CI) | Number of cases | Population Size |
|---|---|---|---|---|---|---|
| Beck, Metz, Svenson & Patten | 2005 | Self-report of doctor diagnosis | Atlantic Canada<br>Prairies, Canada | 350 (230, 470)<br>340 (240, 340) | Not noted<br>Not noted | Not noted<br>Not noted |
| Boström, Callander, Kurtzke & Landtblom | 2009 | Poser | Värmland, western Sweden | 170.1 (154.5, 185.5) | 465 cases | 273,419 |
| Dahl, Aarseth, Myhr, Nyland & Midgard | 2004 | Poser | Nord-Trøndelag County, north Norway | 163.3 (142.2, 187.5) | 208 cases | ~126,797 |
| Ford, Gerry, Johnson & Williams | 2002 | Poser | Leeds, north England | 108.7 (101.2, 116.5) | 792 cases | 728.840 |
| Fox, Bensa, Bray & Zajicek | 2004 | Poser<br>McDonald | Devon, southwest England | 118 (106.1 to 129.1)<br>117 (105.3 to 128.2) | 402 cases<br>399 cases | 341,796 |
| Rothwell & Charlton | 1998 | Poser | South Scotland:<br>Borders<br>Lothian | <br>219 (191–251)<br>203 (192–214) | <br>1613 cases (combined areas) | <br>864,300 (combined areas) |
| Forbes, Wilson & Swingler | 1999 | Allison and Millar<br>Poser | Tayside, central Scotland | 222 (210–240)<br>184 (171–198) | 880 cases<br>727 cases | 395,600 |
| Visser, Wilde, Wilson,Yong & Counsell | 2012 | Poser or McDonald | Aberdeen, north Scotland | 229 (208, 250)[3] | 442 cases | 205,446 |
| Sutherland | 1956 | Allison and Millar(?)[2] | Orkney<br>Shetland | 80 (47, 128)<br>113 (71, 172) | 23 cases<br>25 cases | 40,601 (combined areas) |
| Fog & Hyllested | 1966 | Allison and Millar(?)[2] | Orkney<br>Shetland | 167 (107, 224)<br>120 (69, 170) | 33 cases<br>29 cases | ~18600<br>~17700 |
| Poskanzer, Prenney, Sheridan & Kondy | 1980 | Allison and Millar | Orkney<br>Shetland | 309 (No CI)<br>184 (No CI) | 54 cases<br>34 cases | 17,462<br>18,445 |
| Visser, Wilde, Wilson,Yong & Counsell | 2012 | Poser or McDonald | Orkney<br>Shetland | 402 (319, 500)[3]<br>295 (229, 375)[3] | 82 cases<br>66 cases | 20,000<br>22,656 |

[1] Studies included here resulted from a search of PubMed to identify high-risk areas of high-latitude countries. The search was not systematic or exhaustive; these studies are only to provide some context of how MS prevalence in such high-risk areas of high-latitude countries compare

[2] Not specified in the study but categories of MS described fit the Allison and Millar criteria

[3] Age-and-sex adjusted prevalence estimates (all others are crude prevalence estimates)

For the studies of Orkney and Shetland in Table 4.1, prevalence was defined as the proportion of the population diagnosed with MS in the population at risk (Orkney or Shetland), at a particular point in time. It is clear from Table 4.1 that the prevalence of MS in Orkney and Shetland, despite the difficulties in comparing studies of different regions that were highlighted in Chapter 2, is very high on a global scale. However, it is also important to note limitations in interpreting and comparing the results from the four Orkney and Shetland studies. There are five reasons why the conclusions that can be drawn may be limited.

Firstly, as previously discussed, prevalence is affected by survival. Thus, studies comparing multiple countries that have different health care systems may lead to disproportionate prevalence: countries with lower quality healthcare systems may have lower survival rates and therefore a lower prevalence than countries with higher quality healthcare systems. However, in comparing older with newer prevalence studies, advances in treatment over the years may mean that current prevalence estimates are also an artefact of survival effects rather than an increasing incidence of MS.

Secondly, prevalence estimates depend upon diagnostic accuracy, the precision of which depends on the application of diagnostic criteria to assess whether presenting symptoms are caused by MS. Because the clinical phenotype is variable, application of such criteria will likely vary and have a considerable margin of error (Compston and Confavreux, 2006).

Thirdly, updates to diagnostic criteria may lead to the inclusion or exclusion of individuals who would be differently categorised according to each set of criteria. This was seen in the studies of Fox et al. and Forbes et al. (Table 4.1), who both used two sets of criteria and obtained slightly different prevalence estimates (Fox et al., 2004;

Forbes et al., 1999). The inclusion of MRI data in the McDonald criteria has enabled earlier detection of MS and therefore identifying CIS before conversion to clinically definite MS (Miller et al., 2008), and including CIS in prevalence estimates will be leading to increased prevalence.

Fourth, initial studies of an area are likely to underestimate prevalence, while subsequent studies are likely to identify a greater number of cases as investigator vigilance increases and awareness among the population at risk is raised (Compston and Confavreux, 2006). Intensively studied areas, such as Orkney and Shetland, may therefore not be directly comparable to less studied areas.

Finally, prevalence estimates rely on case ascertainment, which itself often inversely varies with the size and accessibility of the population at risk, particularly as people within smaller, cohesive communities are more likely to be aware of new and existing cases of disease compared to those in large, urban settings (Compston and Confavreux, 2006). The small community sizes of Orkney and Shetland could therefore result in greater identification of cases than in more populous regions.

It should also be noted that the prevalence estimates from the most recent study of Orkney and Shetland are adjusted for age and sex (Visser et al., 2012). The crude, unadjusted estimate is slightly higher in Orkney (410 (95% CI 326 to 509), and slightly lower in Shetland (291 (95% CI 225 to 371)) in comparison with the adjusted estimates presented in Table 4.1. However, it is clear that these prevalence estimates are very high in comparison with other regions and other time periods within the same region.

As described in Chapter 2, rising incidence can also lead to an increasing prevalence. Few studies have explored MS incidence in Orkney and Shetland: such

studies are difficult in small places because of the overall small number of cases and the time that would be needed for sufficient cases to occur. However, those that have studied incidence found that the incidence rate was either stable in both archipelagos (Poskanzer et al., 1980a) or, in Orkney, declining (Cook et al., 1985). However, I also described in Chapter 2 how a disproportionate increased incidence in women over the past decades has resulted in increased prevalence, as well as an increased female-to-male sex ratio (Midgard et al., 1996; Kotzamani et al., 2012; Hirst et al., 2009; Barnett et al., 2003; Alonso and Hernán, 2008; Orton et al., 2006; Ramagopalan et al., 2010a; Wallin et al., 2012). The most recent prevalence study of Orkney and Shetland showed an increasing female-to-male ratio of prevalent cases by birth year (Visser et al., 2012). It is possible that the increasing sex ratio of prevalent cases in Orkney and Shetland reflects an increase in incidence, as it has elsewhere.

Despite limitations, it is clear that Orkney and Shetland have a very high prevalence of MS which appears to have been increasing in recent years. Such high prevalence is of concern in these islands. In this chapter I seek to explore two questions regarding MS in the Northern Isles:

1) Can clusters of MS be identified within Orkney and within Shetland, either in space, in time, or in space and time?
2) Do any identified clusters illuminate potential environmental factors involved in the aetiology MS?

I begin by exploring what is meant by 'disease cluster', and the challenges of establishing with any confidence whether disease clusters exist. I then discuss why disease clusters are interesting epidemiologically, and particularly in the context of the Northern Isles, before summarising MS cluster studies from Orkney and Shetland and elsewhere.

I then describe the methods for investigating potential MS clusters in the Northern Isles and present the results of analyses, and in conclusion I assess some of the challenges encountered in this investigation, and the limitations of the available data to address the research questions effectively. Finally, I make recommendations as to how this question could be more effectively investigated.

## 4.2 Background

### 4.2.1 What is a disease cluster?

The term 'disease cluster' is inconsistently defined in epidemiology. However, when the purpose is to look for a shared environmental aetiology, the implication is that an excess of cases against some background rate should be evident. Clusters resulting from an environmental cause should be specified within boundaries of time, or of space, or of time and space. Temporal clusters refer to fluctuating incidence of disease that follows similar patterns in different places. Spatial clusters refer to an area in which a disease appears more highly prevalent (Rothman, 1990b). Spatiotemporal clusters, however, refer to an aggregation of disease in terms of both time and space and are considered the most revealing for an environmental aetiology, because explanations of the cluster are limited to factors that occur within the vicinity of, and with a temporal relationship to, the apparent disease excess (Rothman, 1990b). However, exactly how to define these boundaries is not clear.

When several cases of disease are spotted together – such as a series of cancers along the same street – it is tempting to search for a common environmental exposure that could be aetiological. Such cluster identification focuses on the occurrence and identification of cases, and does not consider the timing of exposure. In this case, for example, any exposure which may have contributed to the cancer may have happened years prior to disease onset. This period of latency, as well as different periods of latency in different people, means that there is a strong possibility that any apparent

common exposures are merely coincidental, and will not be related to the same disease in other areas (Murray, 2004; Rothman, 1990b).

Further, in small study regions, identifying a population denominator in terms of time and space is complicated by population fluctuations that are not recorded in the decades between censuses. These fluctuations, including emigration and immigration which lead to increasing or decreasing population sizes, can introduce errors that would be cancelled out in larger studies (Elliott and Wartenberg, 2004; Elliott and Wakefield, 2001).

Three further issues also complicate clustering analyses. One of these problems in boundary shrinkage. This is a problem where the identified population from which the cases are supposed to have arisen is too small. As a consequence, this will inflate the numbers in the apparent cluster. To illustrate this point, Rothman (1990b) applied an analogy. He said that defining the cases first and the underlying population second is equivalent to firing a gun and then drawing a target around the bullet hole.

Secondly, the null hypothesis in cluster analyses is complete temporal, complete spatial, or complete spatiotemporal, randomness (Donnan et al., 2005). Statistical analyses to detect clusters invariably involve multiple comparisons of the rate of disease within geographical subregions and the area as a whole (Olsen et al., 1996). Therefore, if we set our family-wise nominal significance threshold at 0.05, and the null hypothesis is true, over the long run we can expect that 1 in 20 comparisons will yield a significant difference (Waller and Gotway, 2004). A more stringent significance threshold would therefore be appropriate to account for the multiple comparisons being performed.

Thirdly, the available data can limit the types of exploration and hamper the conclusions that can be drawn from such analyses. Not only does case ascertainment need to be complete across the region and disease consistently defined, but details of each individual's life are required to enable exploration of exposure histories for illnesses which have long latency periods (Elliott and Wartenberg, 2004). Additionally, further details about individuals' pasts are necessary to control for possible confounding in analyses (Wartenberg, 2001). However, clusters of disease are usually well-publicised, at least within the community, and this renders the collection of unbiased data challenging or impossible as knowledge about the disease and rumours about its cause increase (Rothman, 1990b). Many clustering studies are also hindered by the ecological fallacy. What this means is that differences in healthy and unhealthy regions may not differentiate healthy and unhealthy individuals. Inferences at the group level cannot therefore be applied to the individuals comprising the group (Elliott and Wakefield, 2001).

Investigating reported clusters is therefore complex. Alongside the temptation to 'fish' for common environmental factors, the dangers of retrospectively analysing an area of clustering, chance findings from multiple testing, and difficulties in establishing an accurate dataset, limit confidence in establishing the existence of a cluster. However, despite these potential shortcomings, clustering studies can be epidemiologically interesting.

### 4.2.2 Why investigate disease clusters?

Disease clusters can provide aetiological insight and may be useful in generating aetiological hypotheses. However, they require confirmation by studies from another area or time period (Elliott and Wartenberg, 2004). Clusters are often initially reported by concerned residents who have spotted several cases in their locality, or reported by the media, sometimes following local concern (Elliott and

Wartenberg, 2004; Olsen et al., 1996; Wartenberg, 2001). Such reports are often based on inconsistent and questionable criteria for diagnosis and case ascertainment (Rothman, 1990b). The background population is also likely to be poorly defined (Rothman, 1990b). There is currently no systematic, scientific method for determining when a possible cluster should be investigated, potentially leaving other unusual disease clusters undetected and unstudied. This random approach means that it is difficult to put results from clusters that have been investigated into context, as reports that are investigated could lead to a biased assessment (Wartenberg, 2001). That the investigation of clusters often results from interested and vociferous communities (Cutler et al., 1986; Richardson et al., 1999; Rothman, 1990b) may mean that some clusters are artefacts of close-knit locales.

Cluster analysis is a form of spatial epidemiology and is divided into three main areas: disease mapping, geographic correlation studies, and disease clustering (Elliott and Wartenberg, 2004). One of the most famous examples of disease mapping is John Snow's 1855 work on cholera in London. Mapping the location of cholera cases enabled Snow to narrow down possible causes and eventually identify the aetiological involvement of the Broad Street water pump in the spread of disease (Snow, 1855). Cholera is a good example of how cluster investigations for a disease with a simple aetiology (infectious agent) and short period of latency between exposure and disease manifestation is less complicated than for a complex disease with multiple aetiologies and a long latency period.

Geographical correlation studies have been often used in MS research to demonstrate that a greater number of cases occur in countries that are further from the equator. These studies generated the hypothesis that reduced UV exposure and the concomitant reduction in vitamin D may mean that vitamin D is involved in MS

(Acheson et al., 1960; Goldberg, 1974). This theory that has been explored in numerous studies (Antico et al., 2012; Kriegel et al., 2011; Dobson et al., 2012; Kurtzke, 1975). Geographical correlation studies, do, however, require that the methods of identifying and diagnosing a disease are consistent across all regions and all study populations to ensure comparable results (Elliott and Wartenberg, 2004). Further, ecological studies are hindered by the ecological fallacy. Observations thus made therefore need confirming at the individual level, but are nonetheless useful for hypothesis generation.

Putative disease clusters are explored to investigate potential environmental hazards that may be implicated in a disease aetiology (Elliott and Wartenberg, 2004; Olsen et al., 1996). Examples of such clusters would be the cholera epidemic noted earlier (Snow, 1855), the rate of respiratory disease in communities adjacent to a Thai industrial estate badly affected by air pollutants (Jadsri et al., 2006), and an apparent excess of childhood leukaemia cases around nuclear installations in the UK (Beral, 1993).

Clustering of disease within occupations have identified occupational hazards. Famous historical examples include phossy jaw which presented in the London match girls who worked with white phosphorous (Stockman, 1899). Another such example is squamous cell carcinomas of the skin of the scrotum in chimney sweeps who had been exposed to soot in early childhood (Butlin, 1892).

Whilst some of these examples have yielded causal associations (cholera, respiratory disease and air pollutants, phossy jaw, and cancer relating to soot), the causal relationship in other examples has been more ambiguous (childhood leukaemia and nuclear sites). The reason for this ambiguity is that results across different geographical regions and time were inconsistent, arising from the earlier-discussed complexities involved in clustering analyses. Small numbers of childhood cancers

within each defined geographical area and movement of participants out of the study area additionally led to disparate results and increased controversy.

Clusters can be investigated either *post hoc* or *a priori*. *Post hoc* investigations are designed to identify a common factor which may be causing an apparent cluster whereas *a priori* investigations are hypothesis-led (Elliott and Wakefield, 2001). Developing a hypothesis *a priori* reduces the risk of identifying apparent associations which may or may not be aetiologically involved in a disease. Additionally, understanding why clusters occur is of greater epidemiological and public health importance than the detection of clusters themselves (Rothman, 1990b). However, it can sometimes be difficult to distinguish between *post hoc* or *a priori* methodologies. This is because an apparent sudden rise in the incidence of a disease can be quickly associated with an apparent polluting source *post hoc*, which may then be investigated *a priori* (Elliott and Wakefield, 2001). It is important to note that, whatever the method, clustering studies rarely result in aetiological advances, and consequently some researchers (Rothman, 1990b) advocate the abandonment of cluster studies to reduce the number of false alarms and to direct resources more effectively. Whilst there are many complexities to consider in undertaking and interpreting such studies, exploring disease clusters has the potential to generate new knowledge (Olsen et al., 1996). However, possibly most importantly, clustering studies can help alleviate public concerns about living in an area where a disease has anecdotally been considered highly prevalent (Olsen et al., 1996; Elliott and Wartenberg, 2004; Wartenberg, 2001).

### 4.2.3 Overview of MS clustering studies

Apparent clusters of MS have been explored to attempt to elucidate environmental factors that may be implicated in disease. However, the conclusions that can be drawn from cluster studies are limited by the underlying difficulties of case identification. This problem is outlined by a 1959 survey of an area of apparently high

MS prevalence in Duxbury, Massachusetts. Eight probable MS cases with disability were identified. Although the authors were keen to interpret their finding cautiously and did not attribute the cluster to any environmental pathogen, it later emerged that seven cases had developed MS elsewhere and had moved to Duxbury for retirement (Deacon et al., 1959; Kurland, 1994). The apparent cluster was therefore purely coincidental.

Similarly, investigations into a rural Nova Scotia community where fourteen cases of MS had been reported in the population of 150 found two cases to be muscular dystrophy, one to be amyotrophic lateral sclerosis and another to be myasthenia gravis (Murray, 1976). Of the remaining ten cases several common factors were identified, but these were also common among the rest of the community. However, the author highlights two factors as being potentially aetiologically interesting. Firstly, he notes six of the cases were related in two families. Such familial clustering suggests possible genetic involvement in MS aetiology (Murray, 1976). Secondly, the only period in which the cases had been living within the same vicinity was between 1951 and 1952, and there had been a polio outbreak within this region in 1952. A 1963 paper had described some epidemiological similarities between polio and MS, and had suggested that MS could be a rare manifestation of a common childhood disease (Poskanzer et al., 1963). The possible role of childhood infections has been noted by other researchers in case-control studies (Haile et al., 1982; Andersen et al., 1981; Poskanzer et al., 1980b; Compston et al., 1986) and a retrospective cohort study (Nielsen et al., 2000). However, other case control studies have found no evidence of polio (Poskanzer et al., 1980b) or other childhood infections (Bager et al., 2004) increasing MS risk. Two further cluster studies found disparate results regarding the possible role of infection. A spatiotemporal cluster study in Northern Ireland hypothesised that if childhood infections were aetiologically involved, clustering around the location and timing of outbreaks should be evident. However, a set of 783 cases born between 1901 and 1925

in Northern Ireland failed to find any evidence of spatiotemporal clusters (Ashitey and MacKenzie, 1970).

A more recent cluster study in Tayside, Scotland, used an MS registry containing Poser-defined probable and definite MS cases in the region. MS cases included in the registry were initially identified by a survey carried out in 1996, with each participant giving informed consent. The register was then maintained prospectively, giving a sample size of 772 cases which dated from between 1970 and 1997. Cluster analyses revealed that within these cases, there was a significant temporal cluster of 467 for the whole of Tayside between 1982 and 1995. During these years, the region demonstrated a significant increase in MS incidence compared to preceding years. A secondary spatiotemporal cluster of 42 cases was also identified between 1993-1995 in an area south-west of Perth (Donnan et al., 2005). The scan statistic used by the authors automatically adjusted for multiple testing within each cluster analysis, however, it should be noted that multiple analyses were run and no further adjustments were applied, leading to possible type 1 errors. Additionally, the location of cases used was their place of residence when they were entered into the register of MS cases in Tayside and therefore exposure to an environmental agent involved in aetiology may have happened years earlier in a different location. Although it appears that migration rates for Tayside are very low and so the probability of several cases moving to the region is small, it remains possible that, as in previous studies, the cluster developed by chance. Additionally, MRI was introduced in Tayside in 1990 and neurology services expanded in 1995. The service expansion occurs at around the same time that the peaks in incidence were highlighted by the spatiotemporal scan. It may be that the clusters result, at least in part, from improved case identification within the region in later years. Furthermore, the study endpoint was in 1997 and so the 1982-1995 cluster, and the 1993-1995 secondary cluster, may have been unable to include people who had

experienced symptom onset but had yet to be officially diagnosed before the study's end. This therefore raises the possibility that the detection of a cluster was, instead, the detection of a rise in the incidence of MS. Although there is a possibility that the clusters could result from exposure to a pathogen during a specific time period, no infectious agent was identified (Donnan et al., 2005).

Possibly the most famous MS clustering studies are those carried out in the Faroe Islands. Situated in the North Atlantic at a higher latitude than Shetland, the Faroe Islands have been comprehensively studied for MS cases since 1963. These observational studies identified fewer than expected cases compared to Orkney and Shetland (Allison, 1963; Fog and Hyllested, 1966). Multiple systematic searches failed to identify any cases of MS with a year of clinical onset dating prior to 1943 (Fog and Hyllested, 1966; Kurtzke, 1993; Kurtzke and Hyllested, 1979). However, 24 cases were identified between 1943 and 1960, which, the authors argue, clearly meets the definition of an epidemic: "disease occurrence 'clearly in excess of normal expectancy' and likely to derive 'from a common or propagated source'", taken from the American Public Health Association definition (Kurtzke and Hyllested, 1979). Further, these 24 cases appeared to form two series. The first 14 cases had illness onset between 1943 and 1949, and were at least 10 years of age in 1940 (range 11-45 years). The second series had 10 cases with illness onset between 1952 and 1960. Seven of the cases were below 10 years of age in 1940. The authors concluded that there was an epidemic of MS on the Faroe Islands. They further hypothesised that the 8000 British troops who occupied the islands for five years during the Second World War from 1940 introduced an infectious agent which may have had both a long and short period of incubation, following at least two years of exposure. This theory was supported by a spatial relationship between MS cases and the locations where troops were stationed. It was

also supported by a temporal relationship between the timing of the first MS case and the arrival of the military.

However, these spatial and temporal relationships rest on several assumptions, which have been much discussed and questioned (Poser et al., 1988; Poser and Hibberd, 1988; Compston and Confavreux, 2006). Such assumptions include the arbitrary two-year susceptibility period, which means that the study relies on date of illness onset as being relevant to date of illness acquisition (Poser et al., 1988). This association may be meaningless depending upon the period of latency in each case. Additionally, Kurtzke's study accepted possible incomplete case ascertainment before 1943 as evidence of MS absence in the Faroe Islands, rather than interpreting it more cautiously as absence of evidence (Poser et al., 1988). Poser's reanalysis of the data in fact found additional cases with onset occurring before 1943 (Poser and Hibberd, 1988). Moreover, this reanalysis found that the original study arbitrarily excluded cases and accepted questionable cases. When just one questionable case was changed from case to non-case, the statistical significance of the 'epidemic' disappears (Poser and Hibberd, 1988).

Kurtzke's findings were based on an intensively studied region which, as noted earlier, may lead to increasing case ascertainment as both researchers and residents become more vigilant. Additionally, the use of prevalent rather than incident cases favours survivors. Those who had died before they had been registered into the study are necessarily omitted. However, this omission can result in the appearance of a sudden cluster or an epidemic. Inclusion of people as they are diagnosed may instead show a steady incidence. Importantly, better access around the islands meant that travel to and from hospitals was easier following the Second World War (Poser and Hibberd, 1988), and the arrival of a neurology specialist in 1969 meant that more time

and expertise were now at hand to identify cases (Compston and Confavreux, 2006; Joensen, 2015).

A post-war epidemic of MS was also observed in Iceland (Kurtzke et al., 1982). However, a point of contention is whether this epidemic reflected a genuine rise in incidence or coincided with the arrival of neurology services (Benedikz et al., 1994). As the incidence stabilised several years after the arrival of the neurology services, the cluster may have resulted from increased case ascertainment until the excess of undiagnosed MS cases had been identified, leaving only new cases to be diagnosed (Compston and Confavreux, 2006). However, any stabilisation appears to have been only temporary as, reflecting the trend elsewhere, the MS incidence in Iceland appears to have continued to rise (Sveinbjornsdottir et al., 2014).

The role of canine distemper virus as the transmissible agent for the Faroe Islands clusters was raised as a possible cause of the epidemiology of MS in Orkney, Shetland and Iceland (Cook et al., 1978). It is likely that dogs who arrived with British military officers introduced canine distemper into the Faroe Islands during the Second World War. However, there was no evidence that any identified individual with MS had been infected with the virus. There was also no association between outbreaks of canine distemper and the residential location of cases. Therefore, it was concluded that canine distemper virus was not associated with MS in the Faroe Islands (Kurtzke et al., 1988). The initial identification of the Iceland cluster had also been noted as beginning shortly after an epidemic of canine distemper on the island (Kurtzke et al., 1982). However, there was also a high prevalence of MS in regions of Iceland where there had been no such epidemic (Nathanson et al., 1978). Further cluster studies are summarised in Table 4.2 (for more information see Appendix A).

Table 4.2 Summary of MS cluster studies: theories, findings, and alternative explanations

| Authors | Location | Population size | No. of cases | Theory | Explanations/ alternative theories |
|---|---|---|---|---|---|
| (Campbell et al., 1950) | Berkshire, UK | ? | 6 within 500 yards of each other | High lead content of soil and water | Further research failed to find an increased risk of lead exposure to MS |
| (Eastman et al., 1973) | Mansfield, Massachusetts, USA | 10,000 | 14 | Polluted town water supply prior to the outbreak | Eight of the 14 cases had lived near the polluted water supply; one had not been born during the period of pollution. No other explanations |
| (Campbell et al., 1947) | Cambridge and Derbyshire, UK | 8 veterinary researchers | 4 | Proximity to lamb swayback material, a CNS demyelinating disease in lambs | Other lamb swayback researchers did not develop MS, even when handling the same brain material as the researchers who developed MS |
| (Dean and Gray, 1990) | Key West, Florida, USA | 307 nurses | 7 | Doctors and nurses are at increased risk because of their occupations | No overall increased risk was found in doctors and nurses. Family history of two nurses, or an unknown infection may be responsible for the cluster. Alternatively, these nurses may have moved to Key West after symptom onset |
| (Ingalls, 1986a) | Key West, Florida, USA | 30,000 | 23 | Vermillion ooze from a landfill site close to the hospital, hypothesised to contain mercury | No causal agent was identified |
| (Koch et al., 1974) | Mossyrock, Washington, USA | 450 | 6 cases in 2 families | Unconfirmed smallpox epidemic 50 years prior<br>Or<br>Smelting cinnabar to obtain mercury | No conclusions |

Investigations into many clusters have therefore failed to definitively identify environmental conditions or pathogens that increase MS risk, although the infectious agent hypothesis has led to research which identified EBV as a significant risk factor (see Chapter 2). However, in at least one instance the theories raised by such studies have proven hazardous. Ingalls (1986b), who linked mercury to the Key West cluster, claimed that dental amalgam fillings were responsible for his MS symptoms (Ingalls, 1986b). This claim resulted in many people having their fillings replaced, despite no evidence of any causal association (Murray, 2004). Although clustering studies aim to uncover potential causal mechanisms for disease, this example sets out the dangers of selection and confirmation bias to 'prove' theories that have little or no grounding in evidence.

### 4.2.4 Clusters of MS in Orkney and Shetland

Orkney and Shetland have a high MS prevalence. However, it is less clear whether certain parishes or isles have a disproportionately high number of cases. A 1981 study of MS explored whether cases of MS clustered in Orkney and Shetland based on particular points in patients' life histories. These timepoints comprised birth, certain ages, disease onset, and specific years before onset. A table of all possible pairs of patients was assembled for each identified point in time. Each pair was classified by whether they lived in the same parish at each timepoint, and the length of time that separated the patients' life history point. The null hypothesis was that the distribution of time intervals between pairs of cases in the same parish and pairs in different parishes would be the same. Clusters would be confirmed by a shorter time interval in pairs within the same parish to pairs within different parishes (Poskanzer et al., 1981). This type of analysis ('ridit analysis'), does not utilise p-values but tests a group under study against a reference population to give a measure of relative closeness and a test

of statistical significance. The ridit decreases as clustering increases with anything below 0.5 considered to demonstrate significant clustering.

The results identified three clusters of MS within Orkney by place of residence 21 to 23 years prior to onset and three clusters 2 years prior to onset of disease (Poskanzer et al., 1981). The clusters 21 to 23 years prior to onset were centred in Kirkwall, Westray and South Ronaldsay. The clusters just prior to disease onset were in Kirkwall, Westray and Shapinsay. It appears that patients who developed MS were more likely to live in these same parishes just prior to, or two decades prior to, onset. This finding is consistent with the theory that MS may have two periods of susceptibility, one with a long incubation period and the other with a short incubation period. It is, however, interesting that no clusters were identified in Shetland. It was considered that complete ascertainment had been achieved (Poskanzer et al., 1981), and therefore if an infectious agent or environmental exposure was responsible for the Orkney clusters, this study finds no answers for the high prevalence noted in Shetland.

However, this study is likely underpowered because the number of cases was small. Moreover, designating a year of onset is fraught with difficulties in a disease that may not be recognised and diagnosed for years after symptoms begin (Poskanzer et al., 1981). However, that Kirkwall and Westray appear as significant areas of clustering both two years and two decades prior to disease onset suggests that there may be a common risk factor. One possible alternative explanation is that there may have been a higher genetic predisposition in these districts which led to a greater number of MS cases. The analysis may then have detected clusters at these timepoints purely by chance. We investigate this idea of genetic clustering further in Chapter 5.

Although the infectious agent hypothesis has yielded little in the way of explanations for clusters, it is likely that some environmental factor, or factors, act as a

trigger. In the relatively homogenous environments of Orkney and Shetland, there may be other explanations for the high prevalence. The high-latitude location of the islands means that reduced UV exposure and low vitamin D levels in the population could be more important as causal factors in genetically susceptible individuals. These possibilities are explored further later in the thesis.

To further understand the pattern of MS in Orkney and Shetland, we explored the geographical distribution of disease to test whether the disease disproportionately affects people born in particular parishes or isles. As possible aetiological factors may increase risk from early childhood or even before birth (Gardener et al., 2009; Mirzaei et al., 2011), exploring clustering by place of birth may identify potential early life factors involved in disease (Ashitey and MacKenzie, 1970; Acheson and Bachrach, 1960).

## 4.3 Methods

We first explored the distribution of MS within the isles and parishes that comprise the Northern Isles using two datasets. The first dataset was collected in the 1970s (Poskanzer et al., 1980a), and the second in 2009 (Visser et al., 2012). The 2009 dataset was called the Northern Isles Multiple Sclerosis study; taking the initials we refer to the earlier dataset as NIMS74 and the later dataset as NIMS09. The data from both datasets are limited to age at time of study, sex, and place of birth. NIMS74 originally contained more detailed data, including place of residence between birth and onset of disease, however, these variables were not included in the dataset that we were given.

### 4.3.1 Case definition

The NIMS74 data were collected for a large epidemiological, virologic and genetic study of MS in Shetland and Orkney between 1974 and 1977. Cases were ascertained by interviews with GPs, public health nurses, local MS societies and other

health professionals, as well as from previous MS surveys. Each case was reviewed and evaluated by Professor David Poskanzer, and classified as probable, possible, or not MS according to the Allison and Millar criteria (Poskanzer et al., 1980a). The authors were satisfied that complete case ascertainment was achieved (Poskanzer et al., 1981).

The NIMS09 data were collected for a prevalence study of Orkney, Shetland, and Aberdeen, and for an associated genetic study which ran from 2008 onwards. The prevalence day was 24 September 2009. Cases were identified through GP databases, hospital discharge data from each region's hospital, and a large rehabilitation facility. MS specialist nurse databases in Aberdeen and Shetland were also searched. Further cases were identified from Aberdeen Royal Infirmary lab results for positive CSF oligoclonal bands from 1999 and neurophysiology for abnormal visual evoked responses from 1973. All identified records were reviewed by a neurology research fellow (Dr Elizabeth Visser) and supervised medical student to confirm the diagnosis. Cases were accepted if they met the a) Poser criteria for clinically definite, laboratory-supported clinically definite, clinically probable or laboratory-supported clinically probable MS, or b) McDonald 2001 or 2005 criteria.

For this present study, cases from NIMS74 were those that met Allison and Millar probable and possible criteria. There was insufficient information to classify cases further. Cases that comprised the 2009 prevalence study of MS in Orkney and Shetland who agreed to participate in the Northern Isles Multiple Sclerosis study were included in NIMS09. There was only one crossover case who had been in NIMS74 and was also in NIMS09. For the purposes of these analyses, this individual was included in NIMS09.

Other potential cases were identified from two large, population-based studies, the Orkney Complex Disease Study (ORCADES) and the Viking Health Study Shetland

(VIKING). Permissions were obtained to contact their GPs to verify their MS status. Individuals were added when GPs confirmed that definite diagnoses of MS had been made by consultant neurologists according to McDonald criteria. Individuals that did not have a positive test result for MS were excluded. Additionally, a media appeal for anyone with MS to contact us led to the identification of 26 additional potential cases. However, with no reliable method of verifying their health status, they were not included in the final analyses.

We also excluded cases where we only had death certificate information. In these cases, cause of death was described as 'disseminated sclerosis'. Although this could indicate multiple sclerosis, it could also indicate various other conditions. These other conditions may have had more social stigma attached – such as tertiary syphilis – and were therefore not mentioned by name. However, some of these individuals had been seen and diagnosed by Allison in their lifetime, but were not included in the 1974 study. They had however been included in a later study (Roberts, 1991), and were included in the NIMS74 dataset.

Overall, we had 161 cases that met one of our study criteria. One hundred and five of these cases were born in Orkney and 56 were born in Shetland. Many cases had been entered into a specialist genealogical database, RootsMagic, however the paper files containing the NIMS74 data required computerisation. We entered each case with at least two generations of ancestors; the majority extended back for four or more generations. Where only one generation was recorded, individuals were missing, or there was a mismatch between NIMS74 data and data that we already had, we consulted the Old Parish Records for information before 1855, and the births, marriages and deaths (BMD) index for information after 1855, at General Register House, Edinburgh. Census returns from 1841 onwards also provided valuable

information in tracing families when spellings of names were not standardised and when many Shetlanders were still using the patronymic system, taking the father's given name as their surname, often with the suffix of 'son'.

### 4.3.2 Birthplace definition

We recorded registration district of birth as the birthplace. The reasons for this were twofold. Firstly, the boundaries of the registration districts did not change as much as the parish boundaries. Therefore, when parish boundaries changed, the same registration district was likely to still encompass the same area. Where registration districts did change, we estimated population sizes by taking the percentage difference from earlier censuses and applying it to the registration districts that had conjoined or disjoined.

Secondly, births from the 1970s onwards were more likely to take place in hospitals. In Orkney this meant that the birthplace was most often Kirkwall, and in Shetland, Lerwick. Additionally, high-risk pregnancies were, and are, normally delivered in Aberdeen. In these cases, using birthplace made little sense; taking the registration district of birth meant that it was more likely to capture the area in which the family was living at time of birth, and consequently the time of pregnancy and early childhood. We therefore checked all people with Lerwick, Kirkwall, and Aberdeen births in the BMD indexes to ensure that people noted as having these birthplaces were also registered in these areas.

This method of ascribing birthplace was not without its drawbacks. The use of registration districts made geographical sense in Orkney; the boundaries generally denote island and mainland parishes. However in Shetland, some island and mainland parishes are grouped in the same registration district, thereby losing the natural geographical boundaries of the island parishes. Nevertheless, the use of registration

districts offered a relatively stable method of categorising the geography of the islands over many years.

### 4.3.3 Analyses

The spatial scan statistic (Kulldorff, 2015) has been used in several epidemiological studies to identify clusters of disease in a population (Donnan et al., 2005; Sherman et al., 2014). The null hypothesis is that MS is randomly distributed spatially, temporally, and spatiotemporally. For the purposes of these analyses, as in similar studies (Donnan et al., 2005), we assumed a Poisson distribution for the number of cases in each district. Using the Poisson model, spatial clusters can be detected over a geographical area, temporal clusters over the whole area but within time, and spatiotemporal analyses which take into account both geographical area and time.

The Poisson method uses a theoretically infinite number of circular windows of varying diameters that are centred on the registration districts to evaluate clusters in both spatial and spatiotemporal analyses. While spatial clusters use a 2D window, spatiotemporal clusters use a column which focusses over geographical areas and also extends in time. Temporal clusters likewise use a column that extends in time over the whole study region. The window diameter varies from zero, to a diameter that includes a pre-specified maximum proportion of the population at risk. In these analyses, the population at risk encompasses the whole population who do not have MS supplied from census information and averaged over time. The size of the population at risk in the years between censuses is estimated using linear interpolation. The maximum window diameter was set to include up to 50% of the population at risk with a maximum radius of 10 kilometres. Fifty percent is the recommended percentage as it allows the programme to scan for large- and small-sized clusters (Kulldorff, 2015).

Fixing the window size allows the programme to identify clusters within more defined geographical areas.

For each window location and size, the software calculates the number of observed and expected observations, and then conducts a likelihood ratio test. Statistical significance of the likelihood ratio is tested using a Monte Carlo method. The test statistic was calculated for each replica.

The window location and size with the highest likelihood is considered the most likely cluster, and is assigned a p-value. Statistical significance of a cluster occurs if the true test statistic value is among the 5% highest of the 1000 values generated by Monte Carlo procedure. In this way, it is possible to evaluate clusters of different sizes whilst adjusting for multiple testing (Kulldorff, 2015).

To test for clustering in Orkney and Shetland we treated each island group separately and ran separate analyses using NIMS74 and NIMS09 to compare historical and current data. All analyses were carried out using SaTScan v.9.4 (Kulldorff, 2015).

#### *4.3.3.1 Clustering of MS by birth year and birthplace*

Clustering of MS by birthplace has been explored in military veterans in the United States, and assumes that place of birth is most likely to be the place of childhood and where MS risk is first introduced (Acheson and Bachrach, 1960). Birth dates for cases ranged from 1900 to 1990 (Table 4.3). For spatial analyses, the programme calculated an average overall population size from each census decade population count. The temporal analyses aimed to establish whether there was any significant clustering overall in each island group by birth year. The spatiotemporal analysis took a finer approach to looking within this period to see if clusters were observed in specific areas by specific birth years.

Table 4.3 Birth year ranges of MS cases by dataset and location

| Place and dataset | Range of birth years |
|---|---|
| Orkney NIMS09 | 1932 – 1990 |
| Orkney NIMS74 | 1900 – 1951 |
| Shetland NIMS09 | 1931 – 1981 |
| Shetland NIMS74 | 1901 – 1951 |

## 4.4 Results

Adjustment for multiple testing was automatic within each cluster analysis (Kulldorff, 2015). However, we ran twelve cluster analyses which meant that we needed to adjust for multiple testing over all analyses. Using the Bonferroni method, we corrected for 12 independent tests. We used $0.05/12 < 0.004$ as the threshold for significance. Because the temporal and spatiotemporal clustering analyses, and the spatial and spatiotemporal clustering analyses, were not independent, the Bonferroni threshold is overly conservative. We therefore also calculated Benjamini-Hochberg adjusted p-values. The Benjamini-Hochberg method corrects for the false-discovery rate, and is thus less stringent than the Bonferroni adjustment, all the while still reducing the type 1 error rate (Benjamini and Hochberg, 1995). To correct p-values using the Benjamini-Hochberg method, p-values are first ranked from smallest to largest with the first receiving a rank of 1. The p-value for each significance test is then compared to a critical value equal to (i/m)Q where i is the rank of the p-value, m is the number of tests, and Q is the false discovery rate, usually .25, as it is in this instance. Calculations were carried out using p.adjust in R v 3.4.0.

### 4.4.1 Temporal clustering by birth year

Firstly, we found evidence of temporal clustering in Orkney in NIMS09 and NIMS74 (Table 4.4). The first apparent temporal cluster was of 27 people who had been

born between 1951 and 1960. This means that in our sample, more people with MS were born between 1951 and 1960 than would be expected by chance. A chance distribution would have expected that nine MS cases were born between 1951 and 1960. There was a further cluster of 32 people with MS who had been born between 1912 and 1931. Again, a more even distribution would have expected 20 cases to be born between these years. No significant temporal clusters by birth year were observed in Shetland in NIMS09 or NIMS74.

Table 4.4 Temporal clustering, Poisson model, MS cases

| Data | Total cases | No. in cluster | Cluster years | p-value |
|---|---|---|---|---|
| Orkney MS cases NIMS09 | 53 | 27 | 1951-1960 | 0.001** |
| Orkney MS cases NIMS74 | 52 | 32 | 1912-1931 | 0.005* |
| Shetland MS cases NIMS09 | 26 | 9 | 1942-1951 | 0.227 |
| Shetland MS cases NIMS74 | 30 | 14 | 1922-1941 | 0.68 |

* Survives Benjamini-Hochberg correction
** Survives Bonferroni correction

### 4.4.2 Spatial clustering by birth registration district

We found evidence of spatial clustering of MS by registration district of birth in Kirkwall, Orkney, and in Lerwick, Shetland, in the NIMS09 data. In our Orkney sample, 32 cases were born in Kirkwall. The expected number of cases to be born in Kirkwall between these years is 15. In our Shetland sample, 18 cases were born in Lerwick. However, a chance distribution means that the number of expected cases would be eight. There was no evidence for clustering by birth registration district in NIMS74 (Table 4.5).

Table 4.5 Spatial clustering, Poisson model, MS cases, 10 km window radius

| Data | Total cases | Clusters detected | No. in cluster | p-value |
|---|---|---|---|---|
| Orkney MS cases NIMS09 | 53 | Kirkwall & St Ola | 32 | 0.000015** |
| | | Westray | 5 | 0.905 |
| Orkney MS cases NIMS74 | 52 | Birsay | 6 | 0.417 |
| | | Eday & Pharay | 3 | 0.842 |
| Shetland MS cases NIMS09 | 26 | Lerwick | 18 | 0.00047** |
| Shetland MS cases NIMS74 | 30 | Dunrossness, Sandwick & Cunningsburgh | 8 | 0.229 |
| | | Delting | 3 | 0.830 |

** Survives Bonferroni correction

### 4.4.3 Spatiotemporal clustering by birth year and birth registration district

To investigate further whether there is evidence of temporal clustering in specific areas, we did a spatiotemporal analysis (Table 4.6). We found a significant cluster of 22 cases in Kirkwall in the NIMS09 data between 1951 and 1970. This figure of 22 is significantly more than the number expected by chance, which is five. There were however no other significant results.

Table 4.6 Spatial-temporal clustering, MS cases, 10km window radius

| Data | Total cases | Clusters detected | No. in cluster | Cluster years | p-value |
|---|---|---|---|---|---|
| Orkney MS cases NIMS09 | 53 | Kirkwall & St Ola | 22 | 1951-1970 | 0.00000014* |
| | | Westray | 4 | 1951-1970 | 0.560 |
| | | Sanday, Lady, Cross & Burness | 2 | 1951-1960 | 0.976 |
| | | Birsay, Sandwick | 3 | 1951-1960 | 0.985 |
| Orkney MS cases NIMS74 | 52 | Kirkwall & St Ola | 6 | 1922-1931 | 0.68 |
| | | Eday & Pharay | 3 | 1900-1921 | 0.78 |
| | | Shapinsay | 2 | 1922-1931 | 0.88 |
| | | South Ronaldsay and Burray | 5 | 1912-1931 | 0.92 |
| | | Birsay, Sandwick | 3 | 1922-1931 | 0.97 |
| Shetland MS cases NIMS09 | 26 | Lerwick | 8 | 1942-1961 | 0.413 |
| | | Dunrossness, Sandwick & Cunningsburgh | 3 | 1942-1951 | 0.744 |
| Shetland MS cases NIMS74 | 30 | Dunrossness, Sandwick & Cunningsburgh | 4 | 1912-1921 | 0.352 |
| | | Delting | 2 | 1912-1931 | 0.993 |

** Survives Bonferroni correction

## 4.5 Discussion

Earlier in the chapter, I defined clustering as 'an excess of cases against some background rate'. However, as I shall explain, our results should be interpreted very cautiously as both the identification of cases (numerator), and the population at risk (denominator), had significant problems.

From the analyses we ran, we observed two temporal clusters in Orkney, the first in NIMS74 and the second in NIMS09, which suggested that people born between the years 1912-1931 and 1951-1960 were more likely to develop MS in later life. However, there are four main problems concerning the cases. Firstly, the two datasets use different diagnostic criteria. NIMS74 included people who may not be considered as having MS by the criteria used in NIMS09. It is therefore possible that the cluster observed in NIMS74 would not be significant if more specific and sensitive diagnostic criteria had been applied.

Secondly, the cluster in NIMS09 includes people who would have been diagnosed sometime between 1985 and 1994. However, as the age of onset, taken as the date of diagnosis, for MS usually ranges from 20 to 50 (mean = 34) (Brønnum-Hansen et al., 2004), there could still be people born within these years who were not diagnosed until 2010 or after, which is subsequent to the prevalence day. It is therefore possible that there are individuals who were born shortly after this apparent cluster who had not yet had MS diagnosed by the time this prevalence study was carried out. This means that the cluster may be an artefact of age at diagnosis.

Thirdly, as we are taking year at birth as the basis for the cluster, it is likely that we are looking for an aetiological factor that occurs before birth or in early childhood across the whole of Orkney, but that only affects some individuals. Because we have no further knowledge about when these individuals were diagnosed or about their exposures between birth and diagnosis, we could not test whether many other factors contributed to the apparent increased risk in MS in this cluster. Additionally, the time at which the people in this cluster were likely diagnosed is after the introduction of the Poser criteria. As previously discussed, these criteria are more sensitive and specific

than the Allison and Millar criteria. We observed no significant temporal clustering in Shetland, however this may be attributable to the small number of cases.

Finally, the numerator (cases) comprised people that had MS by the specified criteria, most of whom still lived within the Northern Isles. We were unable to trace people who had been born in Orkney or Shetland, and had later developed MS, but were no longer living in the islands. This means that our sample is inevitably biased, and that the apparent temporal clusters could be artefacts of emigration patterns.

There are also two main problems with the denominator, or the population at risk. Firstly, despite our attempts to identify the population at risk from census information, there remain significant problems with this denominator. I demonstrated in Chapter 3 that the populations of Orkney and Shetland fluctuated over many years and are therefore not stable. However, as the census is only carried out once per decade, the population in the intervening years needs to be estimated. The unstable population indicates that this estimate is unlikely to be accurate. This means that the clusters that were identified in analyses may be artefacts of a poorly-defined population denominator rather than a true cluster.

Secondly, census information includes all people resident in Orkney and Shetland when the census was being collected. This means that the population at risk includes people who were born outside the Northern Isles but moved there in the intervening years. As our numerator only included those born within the Northern Isles, and there was a large influx of people to the Northern Isles during the oil boom years, there are significant problems with this poorly-defined denominator. Such changes in population size and migration and resultant difficulties in defining the population at risk can introduce errors into the clustering analysis (Elliott and Wartenberg, 2004; Elliott and Wakefield, 2001).

We observed two significant spatial clusters in the NIMS09 dataset centred in Kirkwall and Lerwick respectively. This finding could suggest that MS cases aggregate more frequently in Kirkwall- and Lerwick-born individuals than would be expected if there was complete spatial randomness. However, alongside the same problems with the numerator and denominator raised as being problematic for the temporal clustering, this finding is questionable for two further reasons. Both reasons are central to the fact that Kirkwall and Lerwick are the capitals of Orkney and Shetland, and both contain the main hospitals.

Firstly, most people in this period are born at the main hospitals, and although we attempted to overcome the difficulty of place of birth versus place of residence at birth by using registration districts, it is possible that this method was imperfect and that more people were registered in Kirkwall and Lerwick than were actually living there.

Secondly, it is possible that, should the people who were born in Kirkwall and Lerwick have stayed in the vicinity, they would have had easier access to the main hospital where the neurology services are based. Therefore, they may have had more chance of being diagnosed. Again, there was no significant clustering in NIMS74.

Finally, there was an apparent significant spatiotemporal cluster of MS in Kirkwall between the years of 1951 and 1970 in NIMS09. This could suggest that people born between these years in Kirkwall were more likely to develop MS. It seems likely that this spatiotemporal cluster is related to the spatial cluster in Kirkwall, and the temporal cluster in Orkney between 1951 and 1960, both of which were observed in NIMS09. There was no clustering in NIMS74.

However, besides having the main hospital, Kirkwall was also the only parish in Orkney to increase in size between 1891 and 1961 (Figure 3.8). In common with other parts of the developed world at this time, and in Lerwick, Shetland, this increase was largely due to inward immigration from rural parts of Orkney. Therefore, it is possible that people who later developed MS were now living in Kirkwall rather than in their home parishes. In this case, the cluster would be an artefact of the population movement.

As well as these difficulties involving definitions and ascertainment of cases and controls, it is important to note that, as mentioned earlier, diseases with long periods of latency between exposure and onset are more complicated to explore in clustering analyses than illness with short periods of latency. Multiple sclerosis is a complex disease with a period of latency which appears to stretch anywhere between about two and twenty years (Poskanzer et al., 1981). This long period between exposure to a potential causal agent and onset of disease means that multiple other possible exposures will also occur before disease manifests. This then complicates the process of identifying an exposure common to all (or many) cases, and in identifying whether any such common exposure may have causal consequences. Moreover, this lengthy period means that there is a greater chance that people may leave the area before disease onset, and therefore the pattern of the cluster is lost.

It is interesting to note that, like our study, Kirkwall was found to be an area of significant clustering in Poskanzer et al.'s (1981) study, which used a more detailed version of this dataset. However, unlike our study, clustering did not occur by birthdate. Instead the cluster in Kirkwall was found 23 years prior to disease onset and just prior to disease onset (Poskanzer et al., 1981). Similarly significant clustering was found in Westray (Poskanzer et al., 1981). Although registration district of birth

potentially uses the location where exposure to an environmental agent first occurs (Bager et al., 2004; Libbey et al., 2014), many other potential risks may be encountered between birth and age at disease onset (Ebers, 2008; Handel et al., 2010; van der Mei et al., 2003). Therefore, the greater detail of cases' lives would enable greater exploration of environmental risks at different times of life.

Another source of differences in findings between the analyses in this present study and in Poskanzer et al.'s (1981) study may result from differences in inclusion criteria. Poskanzer et al. (1981) were able to separate cases and include only those who had probable MS by the Allison and Millar criteria. Our information was insufficient to separate probable and possible MS cases, and we therefore included both. This inability to separate cases will have led to our inclusion of questionable cases and possibly to the apparent temporal clusters that we observed in Orkney and Shetland. It is reassuring, however, that no other birthdate clusters were observed, in line with the previous study that used the data (Poskanzer et al., 1981). Cases in NIMS09 were classified using the Poser or McDonald criteria which, as previously mentioned, are more sensitive and specific than the Allison and Millar criteria. The difference in diagnostic criteria between NIMS74 and NIMS09 may affect who would be classified as a case, and thereby potentially the presence of clusters.

It is also important to acknowledge, as previously noted, that all clustering studies are hindered by case ascertainment. Whilst it was estimated that case ascertainment was probably about 100% in the NIMS74 data (Poskanzer et al., 1981), several cases were missing from the NIMS09 data. These cases were people who had participated in the almost simultaneous genetic study, but who had chosen not to consent to their details being used for further studies, including the prevalence study. Additionally, other cases have been brought to our attention over the past two years;

however, as there was no reliable method of verifying the health status of these individuals, they were not included in our analyses. This is further compounded by the problem raised earlier, that we were unable to trace people with MS who were born in the Northern Isles and had moved away. Incomplete case ascertainment may bias results by either strengthening or reducing any of the associations seen here. Moreover, there is likely to be an issue with power, as, despite the high prevalence of MS, numbers are overall small, particularly in Shetland. It is more important, however, to be absolutely certain of diagnosis, than to include questionable cases in an effort to increase power.

Finally, the main purpose of cluster analyses is to generate testable hypotheses that may explain the presence of clusters (Rothman, 1990b). Kirkwall was the only area that consistently showed clustering, however no testable environmental hypothesis has been generated. This is because of the limitations of this study and the data, the imprecise knowledge regarding if or when an exposure may have taken place, and, importantly, the many alternative plausible explanation for the apparent clustering in Kirkwall.

## 4.6 Conclusion

Our results highlighted potential statistical spatial and spatiotemporal clusters in Kirkwall in NIMS09, and a spatial cluster in Lerwick in Shetland. However, because of the many problems with our data including biases from inaccurately estimated numerators and denominators, there is a strong possibility that our findings are artefactual. Additionally, we could not rule out many plausible alternative explanations.

To understand more about whether MS clusters within the islands, further information, such as details regarding place of residence from birth to time of diagnosis, and complete case ascertainment, is needed. Such an approach would also

require all Northern Isles births to be traced and their MS status verified, whether or not they still inhabit the Northern Isles. Without these details, and with the multiple problems with our data, we are reluctant to conclude that the presence of significant p-values resulting from our analyses actually denote the presence of true clusters and would advise that our results should be interpreted with extreme caution. The possibility of genetic involvement in MS in Orkney and Shetland is discussed in Chapter 5.

# CHAPTER 5. HERITABILITY AND GENETIC CLUSTERING OF MS IN ORKNEY AND SHETLAND

## 5.1 Introduction

A further form of clustering briefly touched upon in Chapter 4 is genetic clustering. Such clustering studies, usually observed in related individuals in families, provide evidence of genetic susceptibility to disease. Estimating the heritability of complex traits from familiality has a long history and considerable literature has been produced (Polderman et al., 2015; Andreasen et al., 1977; Heston et al., 1966; Berman et al., 1979; Seaquist et al., 1989; Tollånes et al., 2014). Studies exploring familiality, such as the comparison of rates of diabetic nephropathy in diabetic siblings, found that clustering of diabetic nephropathy was evident within families. This finding is consistent with the involvement of heredity in susceptibility to diabetic nephropathy (Seaquist et al., 1989). Likewise, a population-based cohort study of cerebral palsy found that people born into families in which cerebral palsy is present are at an elevated risk. The degree of relatedness was found to be important: the highest relative risk recurrence was observed in twins, and reduced as relatedness decreased. However the study noted that the risk of cerebral palsy remains elevated for as much as third cousins (Tollånes et al., 2014).

The aetiology of complex diseases is multifactorial, involving genetic and environmental factors. Therefore when several occurrences are observed in families, a key question to explore is to what extent genes and the family environment contribute to the trait.

To begin this chapter, I explore definitions of heritability before briefly reviewing the literature on the familiality and heritability of MS. I then discuss an approach which we devised to try to better understand how genetics may contribute to

MS in the highly genetically-structured Northern Isles using family pedigrees and population data. Finally, I present the methods, results and discussion.

### 5.1.1 Heritability

Within populations, phenotypic variation occurs because of differences in genotypes and differences in exposure to environmental factors. Additionally, interactions between genes and environmental exposures may exert influence on the trait of interest (Wray and Visscher, 2008). One goal when exploring phenotypic variation is to determine the genetic effect size; the effect size is a measure of how much individual differences for the trait in the population can be attributed to genetic differences among individuals (Plomin et al., 2013).

Estimates of heritability are calculated from the degree of resemblance between relatives (Fisher, 1919; Falconer and Mackay, 1996; Plomin et al., 2013). It is now common to estimate heritability genomically, which does not require prior knowledge of family pedigrees where markers can be used to estimate relatedness (Yang et al., 2010; Yang et al., 2011). However, classically, heritability has been estimated from known relationships in pedigrees. Methods for estimating heritability include comparing the resemblance between monozygotic and dizygotic twins; resemblance expressed between siblings and cousins; and resemblance between parent-offspring pairs or trios (Falconer and Mackay, 1996; Tenesa and Haley, 2013).

Mixed effects models can also be applied to families or pedigrees to estimate heritability (Visscher et al., 2008; Tenesa and Haley, 2013). Furthermore, parent-of-origin effects can be investigated to see if one sex contributes significantly more to the observed variation than the other (Falconer and Mackay, 1996), and familial relative risk, or recurrence risk, estimates can be calculated (Tenesa and Haley, 2013).

Heritability estimates can be further refined to identify the proportion of phenotypic variance owing to additive genetic effects, known as narrow-sense heritability ($h^2$), and the proportion of phenotypic variance owing to total genetic effects, including the effects of dominance and epistasis, known as broad-sense heritability ($H^2$) (Wray and Visscher, 2008). Because most genetic variance and familial resemblance is attributable to additive effects (Hill et al., 2008; Wray and Visscher, 2008; Tenesa and Haley, 2013), narrow-sense heritability is commonly used for heritability estimates. Offspring receive only one copy of each gene from their parents, therefore most relatives share only one or no copies that are identical-by-descent (that is, from a common ancestor). Full siblings, however, are related in two ways – via their mothers and their fathers – and hence can share two copies of a gene identical-by-descent. Dominance, or non-additive effects, based on sharing two copies, therefore contribute to phenotypic resemblance between siblings but not between parents and offspring (Visscher et al., 2008).

Heritability is an important area of study in the search for the aetiology of complex diseases. However, high heritability does not necessarily mean that a gene or genes with large effects are present (Bochud, 2012; Visscher et al., 2008), rather a number of genes, each conferring a very small effect, usually leads to high heritability estimates. Nevertheless, a study's power for discovering genes associated with disease risk is itself associated with heritability. Therefore, when searching for such a genetic cause for the disease in question, it is advantageous to select a population that has a high heritability. Furthermore, awareness of a disease's high heritability may support the requirement for a study into the genetic determinants of the disease of interest (Smith et al., 2011).

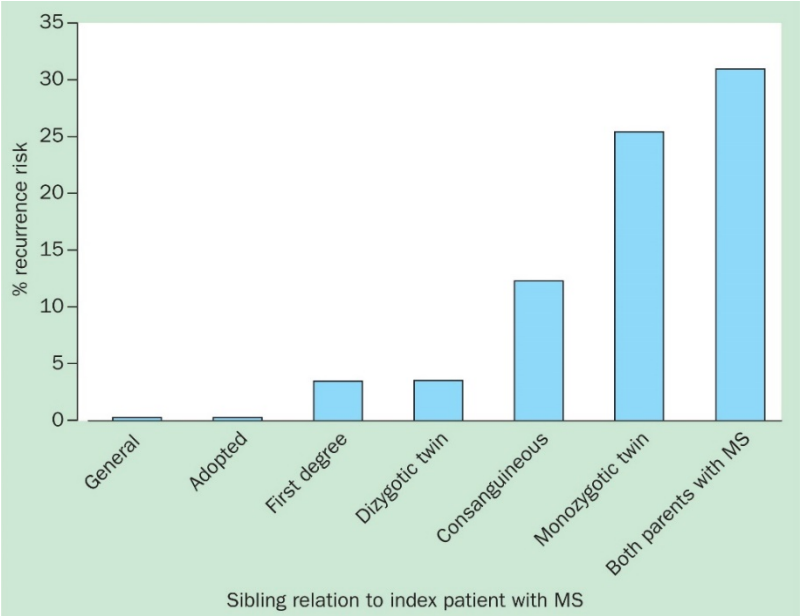
### 5.1.2 Heritability of MS

The observation that MS clusters in families has been widely discussed since the beginnings of the search for risk factors, although historically the idea of a genetic predisposition for MS was not universally supported (Mackay, 1950). However, even from the end of the nineteenth century, the idea that genes may play a role in MS was beginning to take hold. In 1904 Reynolds reported on two families with multiple cases of MS, and suggested that familial cases may be identified more frequently as greater knowledge about MS and its varying forms is accrued (Reynolds, 1904). In 1922 Davenport studied what were then described as 'defects' in draftees in the US Army. He found that the highest rates of MS in the US occurred in the northerly states of Michigan, Minnesota, and Wisconsin, and that people of Scandinavian and Finnish descent had higher rates of the disease. From this study, Davenport suggested that for MS to manifest, some form of endogenous cause, such as 'racial constitution', is essential. He posited that heredity should be considered one such possible endogenous cause, that leads to a raised or reduced risk of MS (Davenport, 1922). This reasoning still failed to garner unanimous support, and in 1930 Brain published a paper questioning the existence of any internal predisposition and instead laid the cause of familial MS – which he still considered to be rare – to the shared familial environment (Brain, 1930). However a 1950 review of all familial MS studies from 1896 to 1948 clearly showed that familial MS was not as unusual as had been believed (Mackay, 1950).

Since then, multiple studies have been produced exploring the familial pattern of MS. A recent study of familial clustering of MS in a Dutch genetic isolate found that 24 of the 48 MS cases could be traced to a single common ancestor in a pedigree of 14 generations (Hoppenbrouwers et al., 2007). Familial studies have also yielded consistent findings showing that age-adjusted recurrence risks of MS increase by

relatedness (Robertson et al., 1996; Sadovnick, 1993; Sadovnick et al., 1988; Carton et al., 1997; Montomoli et al., 2002; Nielsen et al., 2005; Dyment et al., 2004). Figure 5.1 shows how these age-adjusted recurrence risks differ depending upon the degree of relatedness, and that even in more distant relatives the risk is greater compared to the general population (Dyment et al., 2004). Sadovnick et al. (1988) found that this increased risk extends as far as third-degree relatives.

Figure 5. 1 Age-adjusted recurrence risks




(Dyment et al., 2004)

A 1970 review of twin studies found a concordance rate of 15.4% in monozygotic twins and 10.3% in dizygotic twins (Myrianthopoulos, 1970). A longitudinal, population-based study of Canadian twin pairs with MS found a concordance rate of 25.3% in monozygotic twins, 5.4% in dizygotic twins and 2.9% in non-twin siblings (Willer et al., 2003). The increasing MS risk with greater relatedness in these studies, in comparison with the risk in the general population, clearly demonstrates an additive genetic component to the disease; however, it is a fact that most twins are discordant for MS which indicates the requirement of an environmental

trigger to initiate disease in susceptible individuals. This requirement is further reinforced by the difference in MS rates between dizygotic twins and siblings.

Further familial studies reinforced these findings. A study of spouses of MS patients showed that there was no increased risk caused by sharing an environment (Nielsen et al., 2005). Similarly, a study of 238 adoptees living with their adopted family by the age of one found that being raised with an MS patient, when there was no biological relationship, did not raise the risk of MS above that of the general population (Ebers et al., 1995). Studies of half-siblings likewise show that genetic relatedness, and not the familial microenvironment, is the important determinant of familial aggregation. As might be expected, the half-sibling recurrence risk was lower than the full sibling recurrence risk (1.32% and 3.46%, respectively in this study). Moreover, this recurrence risk remained stable regardless of whether the half-sibling had been raised with the MS patient (Sadovnick et al., 1996). Half-sibling studies have also afforded an opportunity to study parent-of-origin effects, with maternal transmission found to confer greater risk (maternal recurrence risk 2.35%, 95% CI 1.57-3.13%, paternal 1.31%, 95% CI 0.65-1.96%, p=0.048) (Ebers et al., 2004). This finding was reinforced by a recent study which found that there was a higher prevalence of maternally-transmitted compared to paternally-transmitted MS (Hoppenbrouwers et al., 2008).

Together, these data provide strong evidence that MS is heritable, possibly more so when transmitted maternally compared to paternally, and suggest that familial aggregation is determined by shared genes and not the familial microenvironment. Such studies have clearly demonstrated the genetic basis to disease, however they do not demonstrate how much of the aetiology can be attributed to genes and to the environment (Dyment et al., 2004).

Several estimates of the heritability of MS have been calculated (Table 5.1). These results range from very modest (15%) (Kuusisto et al., 2008) to high (64%) (Westerlind et al., 2014). A meta-analysis of MS heritability studies indicated that genetics and environmental factors each contribute 50%. The biometric modelling technique applied in this meta-analysis was further able to break down the environmental factors into the effects of the shared (0.21, 95% CI: 0.11, 0.30) and unshared environment (0.29, 95% CI: 0.26-0.33) (Fagnani et al., 2015), the former including factors such as the familial environment, the same school, teachers, community, or peer groups, and the latter different hobbies, schools, teachers, and events such as accidents or illnesses. This is consistent with familial aggregation studies, which suggest that it is not the familial environment, but more likely to be a population-level environmental risk that interacts with the necessary genetic factors to trigger MS. This reasoning is further reinforced by Kuusisto et al. (2008), who note that concordance of MS in dizygotic twins has increased in Finland over the past two decades, however heritability is low. Therefore, environmental factors are likely to be leading to the increasing incidence of MS which is more likely to be population-wide than presenting spontaneously in numerous familial environments.

Table 5.1 Overview of heritability estimates from twin studies

| Authors | Publication year | Study type | Diagnostic criteria | Country | $h^2$ (95% CI) |
|---|---|---|---|---|---|
| (Kuusisto et al.) | 2008 | Twin | Poser | Finland | 0.15 (0.0, 0.77) |
| (French Research Group on Multiple Sclerosis) | 1992 | Twin | Poser | France | 0.25 (0.0, 0.88) |
| (Islam et al.) | 2006 | Twin | Schumacher | North America | 0.31 (0.13, 0.49) |
| (Ristori et al.) | 2006 | Twin | Poser | Continental Italy | 0.48 (0.06, 0.86) |
| (Westerlind et al.) | 2014 | Twin | In the Swedish MS register or ICD code for MS | Sweden | 0.64 (0.36-0.76) |
| (Fagnani et al.) | 2015 | Twin meta-analysis | Poser/ Schumacher | Europe and North America | 0.50 (0.39, 0.61) |

Although a genetic component to disease had been long-recognised, it was not until the 1970s that the *HLA* locus was causally implicated in MS (Naito et al., 1972). As described in Chapter 2, the most strongly related variant to MS risk is the *HLA-DRB1*1501* allele (Oksenberg and McCauley, 2016). However, a study of a large cohort of Sardinian families with MS found that the risk of MS was associated with the *HLA-DRB1*03* and *HLA-DRB1*04* alleles there, and not with *HLA-DRB1*1501* as in Northern Europe (Marrosu et al., 1998). A recent study of familial clustering of MS in a Dutch genetic isolate similarly found that the *HLA-DRB1*1501* allele was not found to be significantly more prevalent in cases compared to controls. These isolates may thus be valuable for explorations into novel genes that raise MS risk (Hoppenbrouwers et al., 2007). Similarly, a lack of association between *HLA* gene variants and MS in Orkney was

highlighted as arising only because the *HLA* haplotypes were present at greater frequency in the Orkney controls than in controls of other regions. In actuality, the frequency of the *HLA* variants in the Orkney cases was similar to that of other Northern European patients with MS (Compston, 1981).

### 5.1.3 Genetic studies of MS in Orkney and Shetland

The island populations of Orkney and Shetland, like the Dutch genetic isolate, are genetically distinct from the larger population. These populations are suited to explorations of genetic susceptibility to disease. The large genetic study undertaken by Poskanzer and Roberts in the 1970s yielded several publications that explored the role of genes in the high prevalence of MS in the islands.

Levels of inbreeding and kinship were assessed to explore the possibility that rare recessive variants and recently-introduced dominant or codominant variants may be leading to an increased risk of MS. Although levels of inbreeding were high in both Orkney and Shetland compared to the rest of the UK, the same levels of inbreeding were found in both cases and controls, and were not high enough to expect any severe effects to general health or mortality. That both cases and controls in both archipelagos are similar in terms of inbreeding suggests that there is no effect of rare recessive variants, however the authors conclude that more common recessive variants could be implicated (Roberts et al., 1979; Roberts et al., 1983).

Similarly, levels of kinship did not differ between cases and controls in either Orkney or Shetland, suggesting that the ancestry of cases and controls is comparable (Roberts et al., 1979; Roberts et al., 1983). The authors therefore conclude that there is no involvement of recently-introduced dominant or codominant variants. However, they note that there may be markers, widely-distributed within the population,

introduced by an individual who made a significant contribution to the gene pool and thus created a founder effect.

However, the polygenic hypothesis, raised within these papers as a way of understanding the genetic contribution to MS in Orkney and Shetland, provides the most likely explanation. This hypothesis fits the patterns of familial aggregation in families of cases compared to controls despite the overall paucity of families with multiple affected individuals, and the declining risk of MS as relationships with the proband, or index case, become more distant (Roberts et al., 1979; Roberts et al., 1983). A tentative heritability estimate was calculated for Orkney, which was 0.47 (95% CI 0.28 – 0.66), however, the low number of cases (51) led to a note of caution in placing too much emphasis on the result (Roberts et al., 1979). No such calculation was attempted for the Shetland sample at it was considerably smaller (31) (Roberts et al., 1983).

It is worth noting that these two above-discussed Orkney and Shetland papers (Roberts et al., 1979; Roberts et al., 1983) obtained their results from analyses of MS cases and two sets of controls. The first set of controls were 'contiguous controls', chosen if they were born within the same parish as the case at around the same time, and lived there for the first 15 years of life. The second set of controls were 'discontiguous controls', and were born around the same time as the matched case but lived for their first 15 years in a non-adjacent parish. However, a follow-up paper in 1991 used the contiguous controls only. This study also included a further 28 patients who had died between 1958 and 1974. These additional patients were included if a) their MS status had been confirmed while they were alive; b) if they had been included in the 1954 series verified by Sutherland (Sutherland, 1956), or c) if they had been in the 1970 series identified by Poskanzer (Poskanzer et al., 1976). Contrary to his

previous study, Roberts found that cases in Orkney were more closely related to one another than were contiguous controls. He also found that the mean kinship of the parents of cases was twice that observed in the parents of contiguous controls (Roberts, 1991). This closer kinship between parents of cases compared to parents of controls would suggest that, at least in Orkney, genes shared by the parents of cases, and genes that are both maternally and paternally inherited, may influence risk of developing MS (Roberts, 1991).

Results therefore vary according to the choice of controls, however there are advantages to using the contiguous controls. As described, contiguous controls were randomly selected from the same parish as the matched case. This geographical matching suggests that, if anything, they were likely to be overmatched on genetic ancestry based upon small community sizes and limited historic population movement. However, the relatively small study sample size of cases and controls also needs to be considered.

Further unpublished analyses from Orkney and Shetland data sought to investigate the relatedness of cases compared to age-matched controls, and to controls who were considerably older and beyond the lifetime risk of developing MS at the time of selection. Using the Northern Isles Multiple Sclerosis Study 2009 (NIMS09) data, the results showed that controls in both Orkney and Shetland were more closely related to each other than cases (personal communication, McQuillan 2016). However, the way in which cases and controls were selected differed on the following important aspects. As described in Chapter 4, cases in this study were identified as part of a genetic and prevalence study of MS in Orkney and Shetland. Considering that complete and accurate case ascertainment for such a study is vital, much effort was directed to these searches. In contrast, controls tended to be volunteers who were interested in their

Northern Isles heritage and were more likely than cases to have all four grandparents from the islands. Families also often volunteered together, or family 'matriarchs' encouraged others in the wider family to join up. The greater Orcadian or Shetlandic ancestry of controls and the potential bias generated by the inclusion of some large families meant that controls were found to be more closely related to each other than the cases (personal communication, McQuillan, 2016). Therefore, no clear conclusions about the pattern of familial MS could be drawn from these data.

### 5.1.4 Genetic clustering: a novel approach

As discussed in Chapter 3, there is strong genetic structuring in Orkney, which is also likely to be present in Shetland. This structuring suggests that geographic regions are a proxy for genetic relatedness. Alongside our deep pedigrees of families with MS, this genetic structuring enabled us to explore whether, historically, the contribution to the modern gene pool of MS from some parishes or isles is excessive compared to other parishes or isles.

For this present study we had one aim, which was to further explore the possible genetic inheritance of MS in Orkney and Shetland using pedigree data combined from our two datasets. We had two objectives to achieve this aim. These were -

> Objective 1 – Using NIMS74 and NIMS09 datasets, obtain an estimate of the heritability of MS in Orkney and Shetland
>
> Objective 2 – Using NIMS74 data only, compare the registration districts of Orkney and Shetland for their genetic contribution to MS, using ancestors of cases and controls to see if some districts historically contributed more the MS gene pool

## 5.2 Methods

### 5.2.1 Heritability

#### *5.2.1.1 Case definition*

These analyses use the same data and case definitions as described in Chapter 4. However, more individuals were included in the heritability analyses than were included in the clustering analysis. This was because an inclusion criterion for the heritability analysis required only that an individual has Northern Isles grandparents whereas the clustering study required individuals to be born in the Northern Isles. In total, we had 182 cases, which met a) Allison and Millar definitions for probable or possible MS, or b) Poser criteria for clinically definite, laboratory-supported clinically definite, clinically probable or laboratory-supported clinically probable MS, or c) McDonald 2001 or 2005 criteria.

#### *5.2.1.2 Birthplace definition*

For the analyses, fine-grained birthplace definitions by parish or isle were not necessary. We did however categorise cases as Orcadian or Shetlandic according to the archipelagos of their grandparents' births. Additionally, we found four individuals who fell into both archipelago categories with equal numbers of Orcadian and Shetlandic grandparents. One-hundred and eighteen cases were considered to be of Orcadian ancestry as they had two or more Orcadian grandparents, and 68 of which were of Shetlandic ancestry, with two or more Shetlandic grandparents.

#### *5.2.1.3 Analyses*

The cases in our study came from an ascertained sample. However, despite our best efforts to create a full and accurate dataset, information regarding case status of some of the older, and deceased, relatives, was unknown and impossible to verify. A censored sample was therefore inevitable. Falconer's method takes into account this type of sample (Tenesa and Haley, 2013) and is the method used for these analyses.

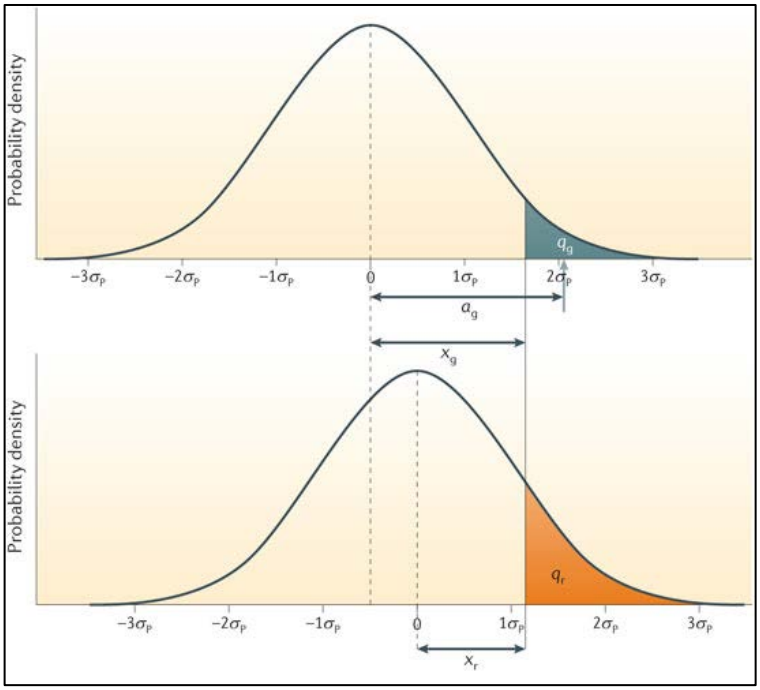
As with other methods, Falconer's method assumes a normally distributed liability for developing a dichotomous trait, such as MS. These models posit that each individual has an underlying risk to disease, which is normally distributed. This normal distribution is termed the liability, and is thought to determine an individual's probability of developing disease (Smith et al., 2011; Visscher et al., 2008). Heritability can be estimated from the liability, and is referred to as the heritability of liability to disease (Tenesa and Haley, 2013).

Falconer's method to estimate the narrow-sense heritability of liability to disease can be applied to groups of relatives of the same structure; we applied it to parent-offspring trios in Orkney and Shetland following the guidelines set out in Tenesa and Haley (2013). The liability of disease is determined from the prevalence of disease within the population; this prevalence determines the threshold of liability above which the disease is expressed, as shown in Figure 5.2.

The population prevalence of MS is denoted by $q_g$, and was taken from the most recent prevalence study of MS in Orkney and Shetland (Visser et al., 2012). The threshold of liability above which disease is expressed is $x_g$. $\Phi$ is the standard normal cumulative distribution, and $\Phi^{-1}$ the inverse of the same. The liability variance, or $V_p$ is assumed to be 1. Then, $q_g = 1 - \Phi^{-1}(x_g)$ and $x_g = \Phi^{-1}(q_g)$. The density of the normal distribution at the liability threshold is denoted $z_g$; the mean liability of index cases ($a_g$) is calculated $a_g = z_g/q_g$. Additionally, $q_r$ is the prevalence of disease among the relatives of the index cases (in these cases, affected parents), and $x_r$ is the normal deviate of $q_r$. The unstandardized regression coefficient of a relative's liability on the liability of an index case is

$$b = \frac{x_g - x_r}{a_g}$$

Figure 5.2 Falconer's method to estimate h2 of liability to disease




(Tenesa and Haley, 2013)

We ran analyses using parent-offspring trios separately for Orkney and Shetland to obtain estimates of heritability for each archipelago. We also calculated 95% confidence intervals for the estimates. Heritability analyses were run using R by David Clark following discussions about the analyses to be undertaken; I calculated the 95% confidence intervals in Excel.

### 5.2.2 Genetic clustering

To explore possible genetic clustering by registration district, we arrived at a sample of ancestors of cases and controls who had been born as close to 1890 as possible, using the NIMS74 index cases and controls. We chose to define a specific time instead of simply looking at the grandparents of cases and controls, as the period in which the grandparents were born spanned over one hundred years beginning in 1801. Trying to define a meaningful population denominator to calculate a proportion of

genetic ancestry for any registration district over such an extended period was therefore impossible. Ancestors born closest to 1890 meant that we could use the 1891 census for the population size.

#### *5.2.2.1 Case definition*

The case definition was as described for the NIMS74 data in Chapter 4. We only used NIMS74 to maintain consistency with regards to controls.

#### *5.2.2.2 Control definition*

As mentioned above, two sets of controls were selected when the data were originally collected in 1974. Contiguous controls were randomly selected from a sampling frame of ten individuals whose dates of birth were closest to the date of birth of the case (either earlier or later), and had been born within the birth parish of the case. Contiguous controls further needed to be unrelated to the matched case, of the same sex as their matched case, had lived in the same area as the matched case for their first 15 years and did not have a known neurological disease (Poskanzer et al., 1980a). As these controls were selected at the time of the study to be temporally and geographically matched to the case, the authors feared that they may have overmatched on an aetiological factor (Poskanzer et al., 1980a). A set of discontiguous controls were therefore also selected. Firstly, all parishes in each archipelago were weighted according to population size taken from the census closest to the birth year of each case, and assigned a number. To assign discontiguous, or non-adjacent parishes to cases, a number was randomly drawn for each case and applied to this list. If a contiguous parish was selected, the process was repeated until a suitable discontiguous parish was found. Once a discontiguous parish had been selected, cases were matched to controls in the same way, and by all the same factors, as contiguous controls.

#### *5.2.2.3 Birthplace definition*

The birthplace definition was the same as for Chapter 4: registration district of birth. However, instead of recording the registration district of the cases and controls as we did in Chapter 4, in this study we noted the birth registration districts for the ancestors of cases and controls, who had been born closest to 1890.

#### *5.2.2.4 Analyses*

Taking the ancestors born circa 1890 of all possible and probable MS cases identified in NIMS74, we noted the birthplace in terms of registration district within Orkney or Shetland and then assigned each ancestor a score based on how related they were to the case: parents = 0.5; grandparents = 0.25, great-grandparents = 0.125 and great-great grandparents = 0.0625. This score was each ancestor's predicted contribution to their descendant's DNA. We then summed these scores for each registration district of ancestors' births, giving a figure that represented the total amount that the ancestors of cases contributed to the NIMS74 MS gene pool in each registration district.

We then divided this figure by the population size of the registration district to correct for the different population sizes. The resultant number was the genetic proportion of the registration district in the 1890s who went on to have a descendent with MS in NIMS74 (hereafter called the genetic proportion).

For comparison purposes, we repeated the procedure with the contiguous controls of the NIMS74 dataset. Case-control comparisons require that the two populations being studied are as similar as possible on all factors except that which is being investigated (CASP, 2016). However, because there is the possibility that the contiguous controls may be over-matched on ancestry, which is crucial to these analyses, we also calculated the genetic proportion for discontiguous controls.

We graphed the results for all registration districts to visualise how the genetic proportions of cases and controls within each registration district compare to each other and across districts. We also calculated 95% CI in each registration district, shown as error bars on Figures 5.3 and 5.5. The null hypothesis was that no registration district would contribute significantly more to the gene pool of modern MS cases than any other. We would therefore expect to see a fairly even distribution of genetic proportion scores across all districts, and across cases, contiguous controls, and discontiguous controls.

## 5.3 Results

### 5.3.1 Heritability

In Orkney, there were 118 cases; of these there were 4 cases who had a parent with MS. We also had 232 non-cases who did not have parents with MS. From these data, we obtained a heritability estimate of 0.36 (95% CI -0.26, 0.98). This means that 36% of the variation in liability to MS between individuals in Orkney is owing to additive genetic effects. In Shetland, we had 68 cases of which there was 1 case who had a parent with MS. We had 135 non-cases who did not have parents with MS. From these data we obtained a heritability estimate of 0.20 (95% CI -1.88, 2.28), meaning that 20% of the variation in liability to MS between individuals in Shetland is owing to additive genetic effects.

### 5.3.2 Genetic clustering

The genetic clustering results showing the genetic proportion of cases and controls in each registration district are shown in Figures 5.3-5.6. Overall, there were no significant differences between cases and controls across all registration districts, shown clearly by the overlapping error bars which provide a range of values in which the true population parameter may fall. This means that no registration district appears to contribute more to the modern MS gene pool than any other.

Figure 5.3 Orkney 1890s population percentage who had ancestors who had MS, or who were selected as contiguous or discontiguous controls (with error bars)

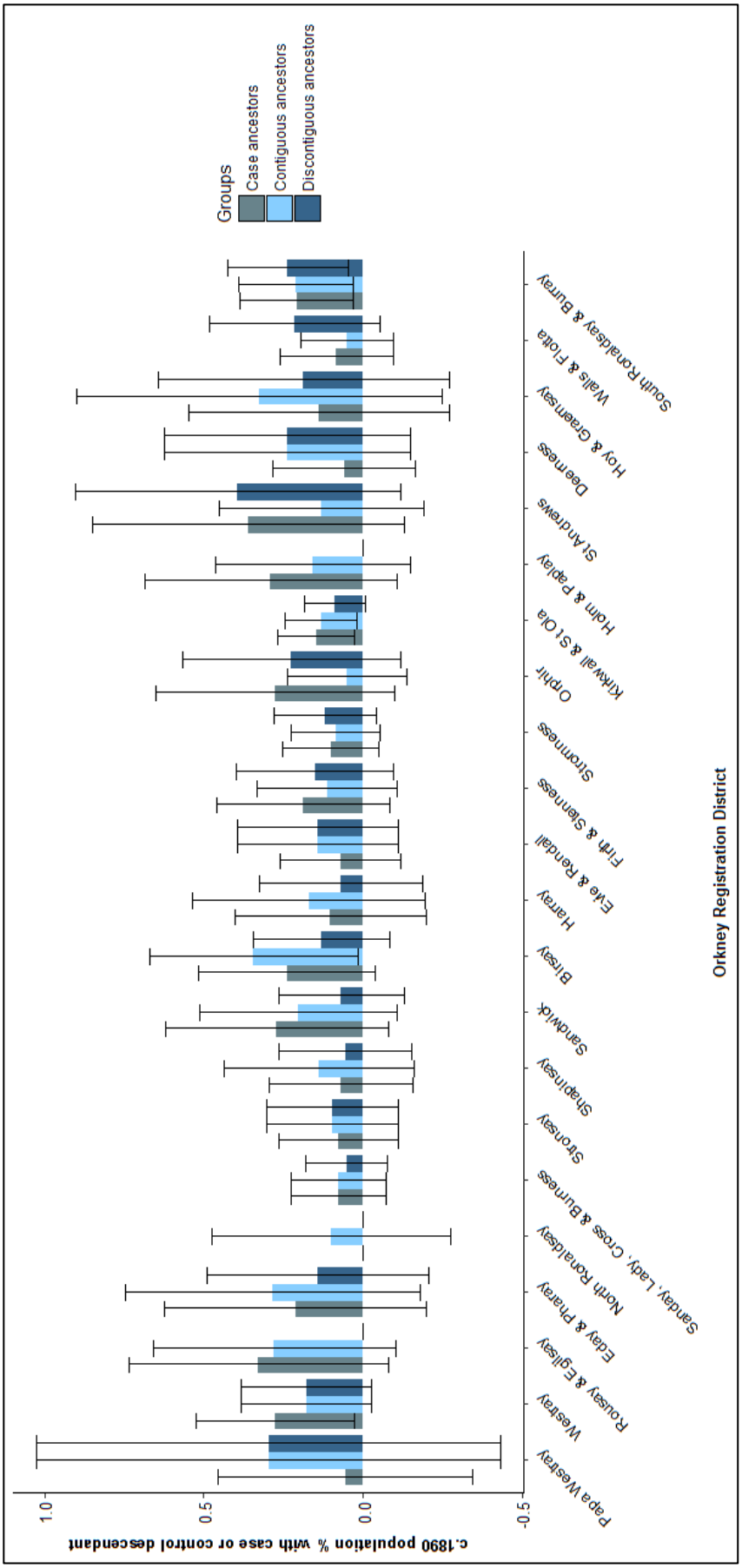


Figure 5.4 Orkney 1890s population percentage who had ancestors who had MS, or who were selected as contiguous or discontiguous controls (without error bars to more clearly show the point estimate)

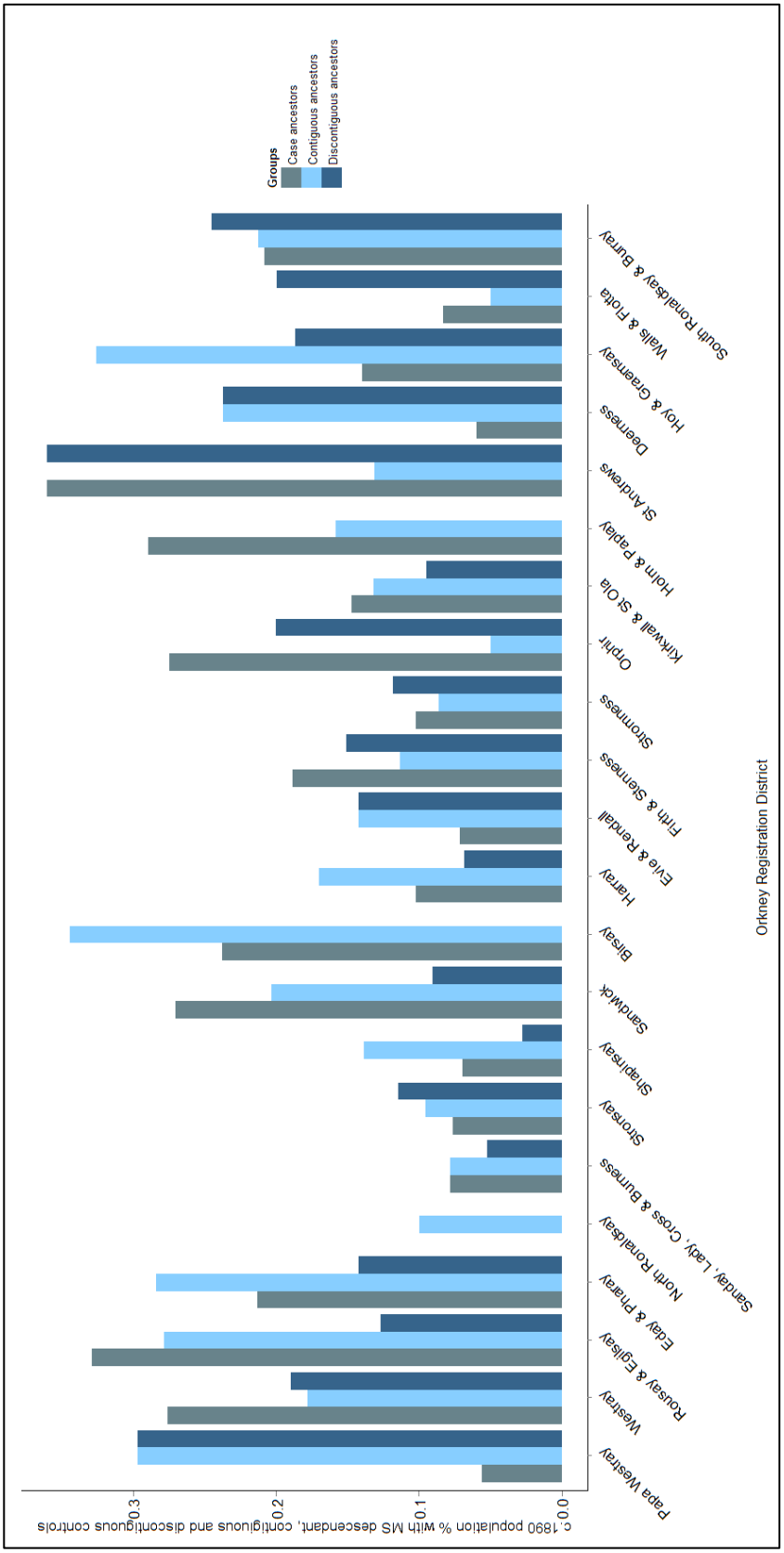


Figure 5.5 Shetland 1890s population percentage who had ancestors who had MS, or who were selected as contiguous or discontiguous controls (with error bars)

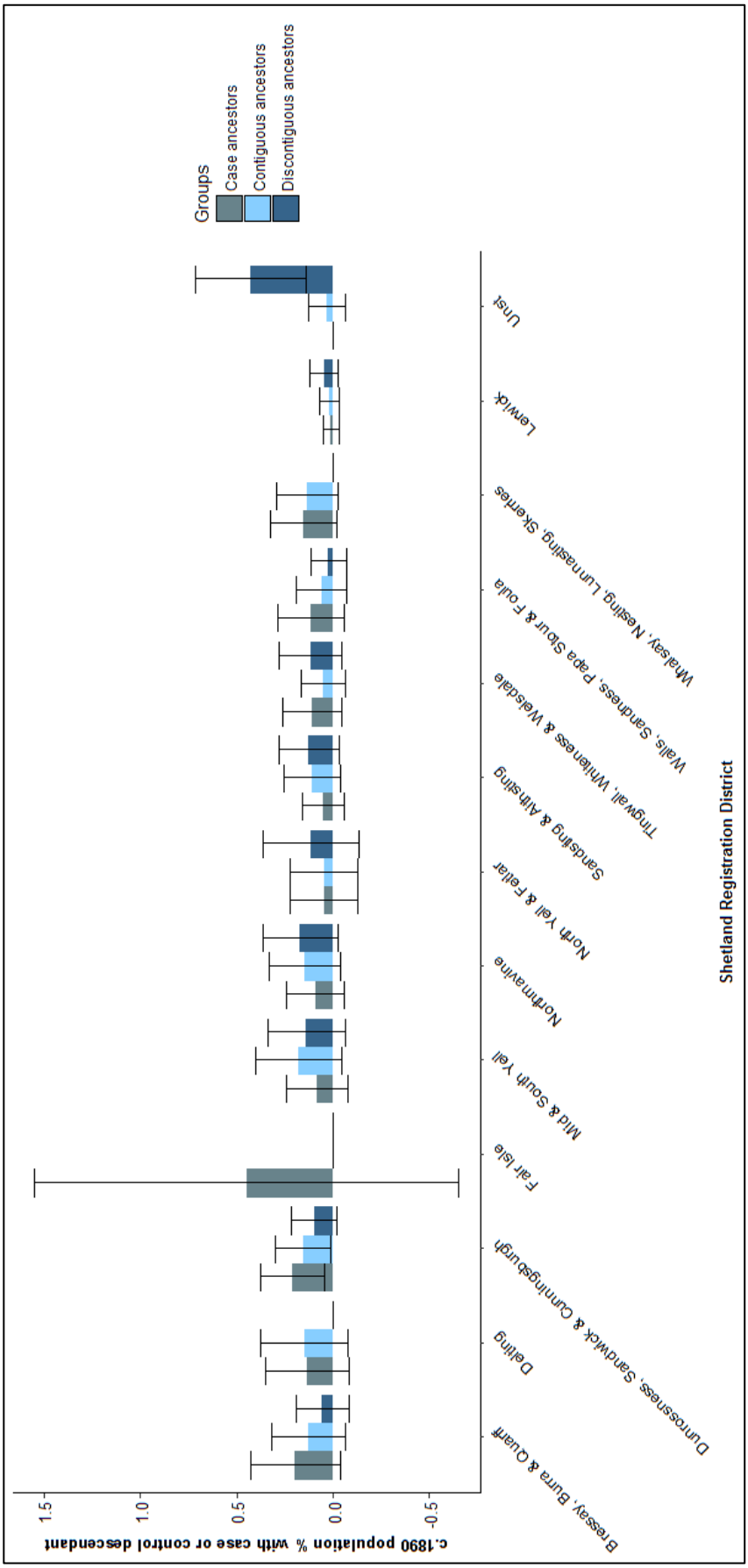
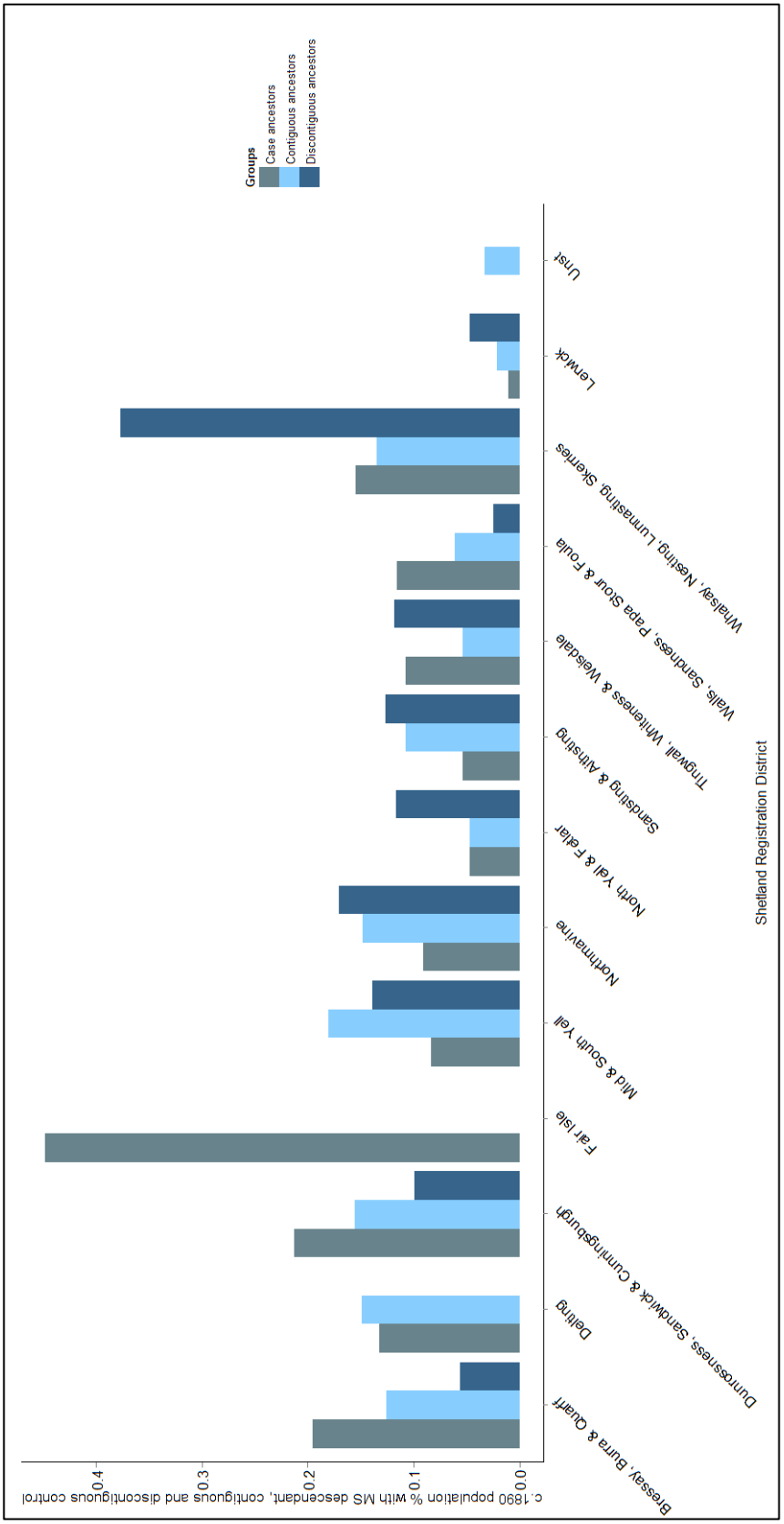


Figure 5.6 Shetland 1890s population percentage who had ancestors who had MS, or who were selected as contiguous or discontiguous controls (without error bars to more clearly show the point estimate)



## 5.4 Discussion

We obtained heritability estimates from parent-offspring trios of 0.36 in Orkney and 0.20 in Shetland with respective confidence intervals of -0.26 to 0.98 and -1.88 to 2.28. These estimates are modest but are consistent with heritability estimates obtained from twin studies outlined in Table 5.1 (Kuusisto et al., 2008; French Research Group on Multiple Sclerosis, 1992; Islam et al., 2006). However, the confidence intervals are large and cross zero. Heritability estimates should fall within the theoretical limits of zero and one; negative confidence intervals for heritability estimates are therefore impossible to interpret meaningfully. In this study, the negative confidence intervals are attributable to the small sample sizes and few related cases in each archipelago, which led to large standard errors. A simulation study which compared heritability estimates from parent-offspring regression with frequentist and Bayesian animal models in wild populations found that small sample sizes in parent-offspring regression had a considerable impact on the precision of the heritability estimates. Moreover, heritability estimates from parent-offspring regression could be negative, particularly when the pedigree on which the analyses were based was sparse (de Villemereuil et al., 2013). However, the decision to base our analyses on parent-offspring trios was taken following consultation with Professor Chris Haley. The decision was driven by our data, which comprised a complex pedigree of multiple families of different sizes and structures, and number of cases too small to effectively explore the heritability using other methods despite such potential resultant inaccuracies. Although these analyses were limited by the available data, the results suggest that genetics are likely to be important in MS susceptibility in Orkney and Shetland as they are elsewhere, although we cannot say that the genetic risk in the Northern Isles is more or less than would be expected.

However, to try to further assess the genetic contribution to today's MS gene pool from the pedigree data by registration district, we explored the genetic contribution of the NIMS74 case and control ancestors. The distribution of the genetic proportions for cases, contiguous controls, and discontiguous controls across registration districts were all similar. This is clearly shown by the overlapping error bars, and therefore we cannot say that any district contributes an excess genetic proportion to the MS genepool.

As mentioned, we were hindered by small sample sizes, both overall and by registration district. This was also problematic in the genetic clustering analyses where some registration districts appeared to contribute a large proportion of genetics to the NIMS74 gene pool (Figures 5.3-5.6). Fair Isle particularly appears to have a large genetic proportion for MS cases. Despite controlling for population size, this is an artefact of a very small population as in actuality, only one father and one mother of an MS case, giving a genetic score of 1 (0.5 + 0.5), came from Fair Isle. However this was enough within that very small population (n= 221 in 1891) to produce a comparatively large genetic proportion. Additionally, none of the selected contiguous or discontiguous controls had ancestors born in Fair Isle, attributable to the even smaller population size of this, the remotest of Shetland's Isles, in the 20$^{th}$ century.

The analyses were carried out within the limits of the data that we had and the controls that had been chosen at the outset of the NIMS09 and NIMS74 studies. To explore the question of the genetic contribution to MS in Orkney and Shetland properly, it would be advantageous to conduct analyses at the genomic level. Other interesting questions to explore would be whether there is a deeper pedigree connection between cases than we were able to detect. As mentioned earlier, Hoppenbrouwer et al. (2007) found a single common ancestor for half the MS cases in their study in a pedigree that

extended for 14 generations enabling the pattern of common ancestry to be established. Despite having deep pedigrees for many of our cases we do not have the same depth for everyone. Additionally, the records dating prior to 1855, when the statutory registration of births, marriages, and deaths was introduced, are less-meticulously recorded. This means that tracing families to these distant generations becomes increasingly complicated or impossible. It is interesting to speculate, however, whether with more complete pedigrees we may see a similar pattern in the genetic isolates of Orkney and Shetland.

Despite these shortcomings, these analyses show that there is a genetic contribution to MS in Orkney and Shetland which reflects the situation seen elsewhere. However, the low heritability estimates indicate that there needs to be a trigger for disease in susceptible individuals. The next three chapter explore such potential triggers, or risks, which are pertinent to these high-latitude islands, including a lack of UV radiation, and vitamin D deficiency.

# CHAPTER 6: A SCOPING REVIEW TO MAP THE LITERATURE CONCERNING VITAMIN D, UV EXPOSURE, AND MS, TO IDENTIFY EVIDENCE FOR AN ASSOCIATION BETWEEN MS ONSET, PATHOLOGY, AND PROGRESSION

## 6.1 Introduction

Chapters 7 and 8 present two primary studies of environmental risk factors for MS. These two studies explore vitamin D levels in Orkney, and UV exposure in Shetland. However, to place these chapters into context, I present in this chapter a scoping review of the literature concerning vitamin D, UV exposure, and MS. I begin with an introduction defining vitamin D and a description of the vitamin D lifecycle. I then explore the best methods and limitations of vitamin D measurement in epidemiological studies. Next, I present a brief history of how the associations between UV exposure, vitamin D deficiency, and MS were initially made and highlight possible mechanisms by which such deficiencies interact with the immune system. Finally, I introduce key study designs in epidemiology and genetics. I discuss in more detail study types that have shown a consistent association between vitamin D deficiency, UV exposure, and MS, and discuss the challenges and complexities involved in inferring causation. I then present the methods, results, and discussion.

### 6.1.1 Vitamin D

Vitamin D is a fat-soluble secosteroid largely formed of two prohormones: ergocalciferol and cholecalciferol (Hewer et al., 2013). Ergocalciferol, or vitamin $D_2$, is of plant origin. Within the diet, mushrooms are a particularly rich source of vitamin $D_2$. Fungi synthesise ergosterols, which, on exposure to ultraviolet B (UVB) radiation from sunlight, are converted into ergocalciferol. Cholecalciferol, or vitamin $D_3$, derives from animal sources including oily fish, eggs, and liver, and is also synthesised from 7-dehydrocholesterol (7-DHC) in skin on exposure to UVB.

UVB is a mid-wavelength light, making up 5-10% of all solar UV. Whilst overexposure causes sunburn, and chronic overexposure can lead to photoaging and increased risk of melanoma and non-melanoma skin cancers (Orteu et al., 2001; Biesalski and Obermueller-Jevic, 2001), UVB also has immunosuppressive properties (Tsunoda et al., 2005) and is responsible for initiating vitamin D synthesis via its action on cutaneous cholesterol. Vitamin $D_3$ from sunlight exposure is the main source of vitamin D in humans (Holick, 2004a).

### 6.1.2 The vitamin D lifecycle

Several different mechanisms enable vitamin D to be produced, activated, utilised, and degraded. Firstly, cutaneous production of cholecalciferol requires 7-DHC, which is produced by the enzyme 7-dehydrocholesterol reductase (DHCR7). 7-DHC reacts with UVB to produce vitamin D. Once acquired, vitamin D undergoes an enzymatic conversion, or hydroxylation, by 25-hydroxylase (encoded by gene *CYP2R1*) in the liver to form 25-hydroxyvitamin D (25(OH)D. This 25(OH)D then undergoes a further hydroxylation by the enzyme 25-hydroxyvitamin D-1 alpha hydroxylase (encoded by *CYP27B1*) to become the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)2D). This second hydroxylation occurs in the kidney, and also in other tissues of the body where CYP27B1 is present. The degradation of vitamin D begins with a further hydroxylation initiated by the enzyme 24-hydroxylase (encoded by *CYP24A1*), which leads to 24,25(OH)2D.

The active form of vitamin D, 1,25(OH)2D, binds to the ligand-binding domain of the VDR, which, alongside another macromolecule, forms a complex at the vitamin D response element (VDRE). This complex results in vitamin D-regulated gene expression (Pike, 2011). The VDR regulates the expression of several hundred genes in over 30 tissues and organs; it is furthermore present in cell membranes where it mediates a variety of rapid responses (Norman, 2006).

For transportation around the body, active or inactive vitamin D binds to vitamin D binding protein (DBP). The main function of DBP is to retain vitamin D and make it available to target tissues. Vitamin D can enter the target cell still bound to the DBP, or it may be delivered and cross the membrane without the DBP. Inactive 25(OH)D can be hydroxylated to 1,25(OH)2D by CYP27B1 when it enters the cell. Vitamin D that has been produced following UV exposure is most quickly bound by DBP (Chun, 2012). Vitamin $D_3$ has also been found to be more potent at raising serum 25(OH)D than vitamin $D_2$ (Trang et al., 1998; Tripkovic et al., 2017), and is considered to be more biologically active (Heaney et al., 2010).

Each part of this chain is vital to the healthy production, activity, usage, and maintenance of vitamin D. A breakdown in any one part can result in deficiency. The different structures of the vitamin D, including inactive, active, and degraded, are all measurable, however some forms are better for assessing vitamin D status than others.

### 6.1.3 Vitamin D measurement

The current best estimate for determining individual circulating vitamin D measures is through analysis of blood for serum 25(OH)D. This inactive form of vitamin D is a robust and reliable marker with a long half-life (Seamans and Cashman, 2009; Mawer et al., 1969), and is not under as tight regulation as the active form (Norman, 2008). Laboratory measurements are however limited in large population studies by logistical considerations which can prevent single, or follow-up, measures being taken. Because vitamin D varies seasonally, single, baseline measurements of vitamin D are not necessarily representative of long-term vitamin D status (Millen and Bodnar, 2008).

Where individual serum measures or longitudinal data are unavailable, dietary data can be used to estimate both recent and lifetime vitamin D (Millen and Bodnar, 2008). Dietary data is however restricted by the need to cover a long enough period to

capture rarely-eaten foods which may be vitamin D-rich. Moreover, it is vital to identify the dose, frequency and duration of vitamin D supplementation which is likely to contribute more to oral intake of vitamin D than diet alone (Millen and Bodnar, 2008).

However, estimates of UV exposure as a determinant of circulating 25(OH)D may be more important in estimating vitamin D status than dietary data (Brot et al., 2001). Methods for calculating UV exposure for a healthy vitamin D status have been developed (Webb and Engelsen, 2006). Latitudes further from the equator have been used as a proxy to indicate reduced UV exposure, which in turn has been used as a proxy for lower vitamin D levels (Holick, 2004a). However, ecological studies such as these do not allow for differences in UV exposure at the individual level.

Satellite data can also be used to estimate UV exposure, and can give a measure of ambient UV for the geographical location and period under study. This method can enable calculation of lifetime UV exposure using information regarding participants' places of residence throughout life (Lucas et al., 2011). However, this calculation still relies on estimates of time spent outside and therefore results in estimates, rather than direct measures, of individual UV exposure.

Other studies quantitatively measure individual UV exposure using polysulphone badges (Webb et al., 1988), other types of dosimeters (Thieden et al., 2004), actinic damage (Lucas et al., 2011), and sun exposure questionnaires to recall recent or lifetime sun exposure (van der Mei et al., 2003). Each of these methods can be applied in combination or used in isolation. Studies that have measured both UV exposure and vitamin D status have explored the direct impact of UV exposure on vitamin D, and also the impact of UV exposure on MS onset independent of vitamin D (Lucas et al., 2011). Several methods of quantifying or estimating vitamin D and UV exposure are therefore available, leading to greater or lesser degrees of accuracy.

### 6.1.4 Key study designs

Several different types of epidemiological study design have been used to explore vitamin D, UV exposure, and MS. Each study design has different strengths and weaknesses, and are ranked in a hierarchy of evidence according to how much weight can be placed on the evidence they can produce. The higher up the hierarchy, the more robust the study (and therefore the evidence) is assumed to be. However, although the weight of evidence that can be placed on studies varies by the study design used, it also depends of the methodological rigour of the individual studies. The hierarchy of evidence contains the following:

1) Systematic reviews of RCTs and meta-analyses
2) Randomised controlled trials
3) Cohort studies
4) Case-control studies
5) Cross-sectional surveys

I present here an overview of the methodologies, strengths, and weaknesses of these study designs. I begin with number 5 on the hierarchy and work through to number 1. I finish with a discussion of the pros and cons of genetic study designs, which are not included in the general epidemiology hierarchy of evidence.

#### *6.1.4.1 Observational studies*

Observational studies are analytic and non-randomised, and involve the observation of study participants with no intervention by the investigators (Sedgwick, 2014). There are three main types of observational study design: cross-sectional, case-control, and cohort (Jepsen et al., 2004). Associations in observational studies result from four main factors. These are bias, confounding, chance, and cause. It is not possible to definitively attribute causation to an association observed in an

epidemiological study, however strong study designs should aim to minimise bias, confounding, and chance, and to carefully assess their effects (Jepsen et al., 2004).

Cross-sectional studies are useful for identifying associations between outcomes and potential exposures or risk factors. However, because cross-sectional studies assemble prevalent rather than incident cases of disease, outcomes and risk factors are identified simultaneously. As such, there is no temporal element in a cross-sectional study and any identified associations may therefore represent consequences, rather than risk, of disease (Jepsen et al., 2004). This also means that the terms 'outcome' and 'exposure' may be misleading, as cross-sectional studies provide no understanding of which presented first.

Cross sectional studies are prone to some biases. Two types of selection bias are non-response bias and volunteer bias. Non-response bias is particularly problematic for studies based on surveys, and occurs when individuals who do not consent to participate in the study differ from those who do participate. This difference can lead to a sample that is not representative of the population (Sedgwick, 2014). Volunteer bias arises from a systematic difference between those who volunteer for a study and the source population. A further bias, ascertainment bias, occurs when the information recorded about participants is systematically different from the truth, and can result from response bias (where participants have systematically supplied information that deviates from the truth), or observer or assessment bias (where investigators systematically record information that deviates from the truth) (Sedgwick, 2015). Confounding, where a third variable obscures an association, can also occur in cross-sectional studies.

Case-control designs select a group with the outcome of interest and a group from the source population, and compare the two for the level of exposure. This study

design is particularly useful for rare diseases, however where the frequency of an exposure is either very common or very uncommon in the population, case-control studies may be limited in detecting associations with precision (Koepsell and Weiss, 2014). Typically, the power of case-control studies to observe an association increases with a higher ratio of controls to cases, up to 4:1 (Wacholder et al., 1992; Hennessy et al., 1999).

Biases particularly relevant to case-control studies include selection bias and recall bias. Selection bias occurs when any apparent association between the exposure and the outcome is combined with the association of being selected as a case or control and the exposure, and can bias either towards or away from the null (Rothman et al., 2008). Recall bias can occur when current health status affects recall of past exposures; those in the case group may recall past exposures which appear more salient than those in the control group (Jepsen et al., 2004). Nested case-control studies are set within a cohort study, where cases have an outcome of interest, and controls are selected from individuals who were at risk at the time that the cases occurred (Szklo and Nieto, 2012). Nested case-control studies reduce selection bias as both cases and controls are drawn from the same population, and recall bias is also reduced compared to a case-control study. There are also methods to reduce confounding. One possibility is to restrict the sample so that people with, for example, a comorbidity that could affect the outcome, are excluded. Statistical adjustment can also reduce confounding where differences between cases and controls have been identified. Finally, risk of confounding can also be reduced by matching cases to controls on a potentially confounding factor (Koepsell and Weiss, 2014).

Cohort studies follow up a group, or cohort, to determine the occurrence of an outcome, and identify how natural levels of exposure may differ in relation to the

outcome. Where follow-up of participants has occurred prior to the start of the study, the study design is retrospective; where follow-up occurs into the future, the design is prospective. For rare diseases particularly, cohort studies require a large study population and a follow-up long enough to observe sufficient outcomes associated with exposures to reduce the risk of chance findings (Jepsen et al., 2004). In cases where a latency period between exposure and outcome is lengthy, cohort studies are impractical to detect associations. Although cohort studies have a temporal element and therefore enable the identification of an exposure that precedes onset of disease – one of the principles of inferring a causal relationship – the association is not necessarily causal. Like the other studies discussed, cohort studies can be influenced by non-response bias and selection bias (Sedgwick, 2011). Furthermore, loss to follow-up can be particularly problematic, and can occur if individuals die, move away, or withdraw consent to continue in the study. These losses can be related to the exposure, the outcome, or both, and can lead to significant biases in the measurement of both exposure and outcome. Furthermore, cohort studies are prone to confounding, as confounding variables may be unavailable or difficult to collect across the whole study period (Hennekens et al., 1987).

#### *6.1.4.2 Intervention studies*

Intervention studies, unlike observational studies, involve the intervention of the researcher. There are several types of intervention study, including non-blinded trials, where both participants and researchers know what treatment is being administered, non-randomised trials, where participants are allocated to an intervention in a way that is not random, and randomised controlled trials (RCTs), which are considered the gold-standard of intervention studies (Bothwell et al., 2016) and are second in the hierarchy of evidence.

In a successful RCT, the randomisation process means that the groups under study are the same for measured and unmeasured factors, which reduces biases and confounding. At its most basic, the investigator compares a treated group with an untreated group, and observes the differences in, for example, disease progression. The controlled setting of the RCT, where ideally the only difference between groups is the administered treatment, enables evaluation of an intervention without the same noise found in observational trials (Thiese, 2014; Nardini, 2014).

Nonetheless, RCTs are subject to complications, including selection bias, where the groups under study differ from each other at baseline in salient ways which can introduce confounding and bias results; performance and detection bias, which refer to systematic differences in the ways in which the groups are treated and in how the outcomes are treated respectively, and attrition bias where there is a systematic difference in withdrawals between the study groups leading to incomplete and biased outcomes (Kovesdy and Kalantar-Zadeh, 2012). There are ways to deal with such issues, including methods for randomisation, masking study participants and investigators to the treatment status of each group, known as double-blinding, and performing analyses that take into account participants lost to follow-up (Kovesdy and Kalantar-Zadeh, 2012).

RCTs of potential new therapies usually include a placebo group – that is, a group that is the same in all ways as the group receiving treatment, but instead of the treatment are given an inert substance. This enables researchers to detect whether the treatment has an effect. However, where an effective therapy is in existence, it is usually considered unethical to deprive a group of treatment. In these cases, it is more usual to compare one group taking an existing treatment with another taking a new treatment (Gupta and Verma, 2013). There is currently insufficient evidence to justify

vitamin D therapy as a routine MS treatment, and therefore placebo groups are valuable in vitamin D therapy trials.

#### *6.1.4.3 Systematic reviews and meta-analyses*

There are multiple types of review study, however systematic reviews of RCTs alongside meta-analyses are placed at the top of the hierarchy of evidence. A meta-analysis is a statistical technique that combines results from multiple quantitative studies, identified by systematic and exhaustive searches, with the aim of giving a more precise estimate of the effect than single studies in isolation (Grant and Booth, 2009). A systematic review, which can include a meta-analysis, is likewise the result of a systematic search for literature to identify all evidence on a subject. Systematic review research questions are usually very well-defined and narrow (Arksey and O'Malley, 2005). Evidence sought on a subject is appraised and synthesised to obtain a full and impartial view of existing literature to answer the question. Findings may be used to understand what research has been undertaken, what uncertainties remain about findings, what is still unknown, and areas for future research. Systematic reviews should adhere to strict guidelines, and both meta-analyses and systematic reviews involve quality assessment of identified studies to determine inclusion or exclusion (Grant and Booth, 2009). The comparison of multiple – quality – studies in a systematic review lends this design its strength.

Systematic reviews have historically focussed on RCTs, and collate evidence for healthcare intervention effectiveness in areas such as prevention, treatment, and rehabilitation. Such a rigorous review of the literature can guide policy-makers and hasten implementation of effective therapies into routine treatment. Such systematic reviews of RCTs are ultimately at the top of the hierarchy of evidence. However, clinical trials are not always feasible or ethical; they may omit long-term, potentially serious,

outcomes, or they do not use real-world settings which can impact their external validity (the extent to which findings can be generalised to populations and situations outside the study) (O'Neil et al., 2014). Therefore, systematic reviews of healthcare interventions using observational studies are becoming more prominent (O'Neil et al., 2014). Although identifying causal associations from observational studies is not possible, and therefore a systematic review of observational studies cannot draw causal conclusions, the collation of several observational studies from different populations in which chance, bias, and confounding have been minimised has led to some observational systematic reviews with strong evidence for further exploration. This is particularly true when findings across multiple studies have shown similarly strong associations and dose-response relationships. Systematic reviews of observational studies, whilst still retaining the same risk of bias and confounding as observational studies, can in certain circumstances therefore be valuable in filling gaps left by systematic reviews of RCTs (O'Neil et al., 2014). Furthermore, such observational systematic reviews can highlight the prevalence of disease and increased risk of comorbidities, both of which can guide future research (Etminan et al., 2005; Jeon and Murray, 2008).

#### *6.1.4.4 Genetic studies*

Genetic epidemiology aims to establish the presence of genetic components for disease, and the genetic effect size or penetrance, which contributes to disease in comparison with environmental causes. In so doing, such genetic risks can be used for several purposes: to predict who is at risk for disease with the possibility of offering targeted interventions; to better understand the biological basis for the genetic effects which may lead to pharmacological developments, and to help identify causal pathways between exposures and outcomes (Bush and Moore, 2012; Smith and Hemani, 2014). Linkage analysis, where genetic markers across the genome in affected families are

examined to understand how the genes segregate in particular diseases, was initially successful in identifying variants that contribute to rare, single gene disorders, such as cystic fibrosis which have a highly penetrant, Mendelian pattern of inheritance (Bush and Moore, 2012; Marian, 2016). However, this approach has been less successful in identifying the genetic basis of common disorders, suggesting that the genetic architecture of rare and common diseases is different. The common disease/common variant hypothesis states that common disorders are likely to be influenced by genetic variation that is common in the population; which do not have Mendelian patterns of inheritance but may aggregate in families, and each of which may have very low penetrance (Bush and Moore, 2012; Marian, 2016). As such, each variant may contribute a small amount of increased disease risk, but there is no guarantee that carrying risk alleles will lead to disease manifestation. Such diseases, like MS, therefore likely manifest as a result of multiple genetic (and environmental) risks, each with a very small effect, but that together create an increasing genetic risk for disease.

Finding evidence of such genes and their role in disease comes largely from genetic epidemiology studies (Marian, 2016). Various study designs have developed in this area of research. Two of the most common designs which I discuss here are genome-wide association studies (GWAS), and candidate gene studies. I also discuss Mendelian randomisation (MR) studies, which use genetic markers as a proxy for environmental exposures.

The GWAS technique was first validated in a landmark 2007 paper (The Wellcome Trust Case Control Consortium, 2007). They are non-hypothesis driven, exploratory studies which can be performed for both binary and continuous traits, and are used to identify common variants in the genome with small or moderate effects (Bush and Moore, 2012). GWAS have been performed using cohort designs, where a study population is selected and prospectively followed for the development of

conditions, which also enables interaction effects between genes and environment to be investigated in detail (Manolio, 2009). However, typically, GWAS use case-control designs, where common genetic variants are evaluated for an association with disease using affected and unaffected individuals. Some GWAS use family designs involving affected and unaffected family members, which controls for potential confounding by shared environmental exposures. Such a design additionally removes the possibility for population stratification bias, which arises from differences in genetic ancestry between cases and controls, although it also reduces power to detect main effects due to the increased genetic similarities between family members (Witte, 2010).

As with all case-control designs, control selection should be representative of the source population (Witte, 2010). Convenience controls, where genotype information from controls who were involved in previous studies is used for control populations in current studies, are also used (Luca et al., 2008). The definition of the phenotype according to standardised criteria is also important, particularly when a study is based across multiple centres (Bush and Moore, 2012). However, recall bias, which can hamper traditional epidemiology studies, is not relevant to genetic studies, and large sample sizes also add strength to the design (Witte, 2010).

GWAS conduct millions of tests across the genome in an effort to identify potential genetic risks. Each test has its own false positive rate and adjustment for multiple testing is therefore crucial (Bush and Moore, 2012). Various methods have been applied to correct for multiple testing, including the False Discovery Rate, permutation testing and genome-wide significance (Bush and Moore, 2012). However, the gold standard in confirming an identified effect is to replicate it in an independent sample (Bush and Moore, 2012) and this is where candidate gene studies are particularly useful.

Candidate gene studies are association studies where the aim is to identify the presence of a statistically significant association between specific genomic variation and disease. Genes are chosen based upon a biologically plausible hypothesis which may result from animal models, or from prior knowledge of the effect of genes on other traits (Hattersley and McCarthy, 2005). Today, candidate genes are often identified from significant results in GWAS and undertaken to replicate the observed effects (Bush and Moore, 2012). Like GWAS, such studies can be case-control, family-based or within-cohort; again family-based designs reduces some possible environmental confounding factors and are particularly useful in identifying rare variants (Zondervan and Cardon, 2007). As in traditional epidemiology, cohort studies are considered to be a very strong design (Manolio, 2009), although when a disease is rare the numbers of cases are likely to be low, and any resulting nested case-control studies are therefore likely to be small. Case-control designs are again the most widely used (Zondervan and Cardon, 2007).

When candidate gene association studies are undertaken to replicate an association observed from GWAS, the first aim is to reproduce the effect in an independent dataset from the same population as the GWAS. If the effect is replicated and confirmed, then a different population is targeted to identify whether it is population-specific or whether it is relevant to multiple human populations (Bush and Moore, 2012). Where the analysis is hypothesis-driven, the number of tests should be small and adjustment for multiple testing is not required to the same level as GWAS (Bush and Moore, 2012).

Replication has however been a frustratingly slow process, which may be attributed to several potential problems that limit ability of such studies to replicate findings. Firstly, because the effect detected in a GWAS may be stronger than in the

general population, a candidate gene study sample size needs to be large enough to have the power to detect an effect which is likely to be smaller than anticipated, or to detect whether the initial result was spurious (Bush and Moore, 2012). The phenotype must also precisely adhere to an accepted standard; slight differences have the potential to prevent replication (Bush and Moore, 2012). Additionally, sample and variant selection, genotyping error, analytical techniques, and interpretation may reduce the ability to replicate observed associations (Hattersley and McCarthy, 2005). As well as potential biases within studies, publication bias may also be present, where only a small amount of the most 'publishable' (or significant) findings are to be found within the published literature. Findings which had not been replicated are thereby omitted from the evidence base, which could mean that the significant results reported are no more than chance findings (Ioannidis, 2003).

Replication is important in confirming an association, and also in defining whether there is a potential causal pathway between an identified gene and the phenotype of interest. However, well-conducted Mendelian randomisation studies offer possibly the best evidence to evaluate a potential causal association.

Mendelian randomisation studies seek to answer different questions to genetic association studies. As described above, genetic association studies aim to identify genes associated with complex traits. However, Mendelian randomisation studies use genetic variants as a proxy for modifiable environmental exposures. The basic idea underpinning Mendelian randomisation is that if a modifiable environmental exposure is related to an outcome, then a genetic variant that either alters the level of, or mirrors, the same exposure should be related to disease risk (Smith and Hemani, 2014). The random allotment of alleles in meiosis (the process of cell division where pairs of chromosomes divide), is analogous to the randomisation of participants in an

RCT. Mendelian randomisation studies may therefore be viewed as 'natural' RCTs (Thanassoulis and O'Donnell, 2009), and it is this randomisation which protects Mendelian randomisation studies from many sources of bias and confounding. By using functional genetic variants as proxies for environmental exposures, Mendelian randomisation has several advantages over traditional epidemiology designs as genetic variants are not affected by behavioural, cultural or physiological factors that can confound associations; they are fixed from birth and so reverse causation and measurement error are excluded, and reporting of variants cannot be modified according to knowledge of disease status (Smith and Hemani, 2014). Additionally, genetic variants may act as long-term environmental exposures (Smith and Hemani, 2014), for example a variant that lowers production of vitamin D can be considered equivalent to long-term, or lifetime, vitamin D insufficiency.

As with genetic association studies, problems with Mendelian randomisation can occur when the biological association between genotype and phenotype is not accurate and reliable. Furthermore, confounding can occur as a result of population stratification (differences in allele frequencies in cases and controls resulting from systematic differences in ancestry (Springer, 2006b)) or linkage disequilibrium (the non-random association of alleles at different loci within a population (Springer, 2006a)) of one functional variant with another, and pleiotropy, where the gene in question has multiple functions and phenotypic consequences and may therefore be acting on a different pathway to increase disease risk (Smith and Hemani, 2014). However, if it is concluded that there is no alternative way in which the variant could be affecting the outcome, then the Mendelian randomisation design provides strong evidence for causality which may have implications for the manner in which disease is prevented at the population level (Smith and Hemani, 2014).

There are therefore multiple study designs, which are increasingly sophisticated, aiming to establish the association and direction of causation between vitamin D deficiency and MS. However, the link between UV radiation, vitamin D deficiency and MS, was first spotted from ecological studies, where associations between a population (rather than individuals) and the outcome of interest, are identified.

### 6.1.5 The association between vitamin D, UV exposure, and MS

The association between MS and UV exposure was first documented in 1960 (Acheson et al., 1960). This observation followed the recognition of a pattern where MS prevalence increased as sunlight decreased resulting from distance from the equator. Additionally sunnier local climates were observed to be protective against MS (Acheson et al., 1960).

Soon after this observation, sunlight's role in initiating cutaneous synthesis of vitamin D was established (Rauschkolb et al., 1969), and a hypothesis was formulated for the aetiological involvement of vitamin D in MS (Goldberg, 1974). The role of vitamin D in the immune system was first identified in the 1980s (Bhalla et al., 1984), and in the late 1990s 1,25(OH)$D_3$, the active form of vitamin $D_3$, was found to prevent the onset of experimental autoimmune encephalomyelitis (EAE), a mouse-model of MS (Hayes et al., 1997). The role of vitamin D in *VDR* gene expression is also now hypothesised to have a significant role in large gene-gene interaction systems. These interactions may regulate immune processes that modulate MS activity, shown through greater MRI activity in vitamin D-deficient individuals (Munger et al., 2014).

Also in the late 1990s, a hypothesis was proposed suggesting that the pattern of MS prevalence may reflect differential UV-induced autoimmune activity suppression (McMichael and Hall, 1997). The action of UV independent of vitamin D has also been

shown to reduce inflammation and demyelination in EAE (Becklund et al., 2010; Wang et al., 2013; Wang et al., 2015).

Both vitamin D and UVR similarly affect the activities of autoimmune-active dendritic and $t_{reg}$ cells (Hart et al., 2011). Furthermore, sun exposure and vitamin D were found to be independent risk factors for CNS demyelination in a multicentre Australian case-control study (Lucas et al., 2011).

Since the early recognition of a geographical pattern, many studies have continued to find increased MS prevalence with distance from the equator in both northern and southern hemispheres (Behrend, 1969; McCall et al., 1968; Acheson and Bachrach, 1960; Simpson S. Jr. et al., 2011; Hornabrook, 1971). The most influential studies are perhaps those of Kurtzke, who proposed the existence of a latitudinal gradient with geographical low, medium and high MS risk zones corresponding with distance from the equator (Kurtzke, 1975; Kurtzke, 1977; Kurtzke, 2000). High risk areas included most of Europe, the northern states of America, Canada, New Zealand, south-east Australia and east Russia; medium risk areas comprised the southern states of America, South Africa, the southern Mediterranean basin, the rest of Australia, parts of Latin America, the Ukraine and Russia into Siberia, and low risk areas consisted of Asian, African and northern South American countries (Kurtzke, 2000).

This geographical pattern has been consistently observed in the literature. Notable exceptions, however, are Sicily and Malta. Situated at the same latitude, the two islands have a 10-fold difference in MS prevalence with Sicily being a high-risk area and Malta very low-risk (Dean et al., 1979; Vassallo et al., 1979). Follow-up studies found this difference to be enduring (Nicoletti et al., 2011; Dean et al., 2002). Plausible reasons for the disparity could be genetic and environmental differences which either lead to an increased risk in Sicily, or are protective in Malta. However, studies on these islands to date have yielded no explanations (Dean et al., 2008).

Migration studies also support the latitudinal gradient of MS risk. This effect was first noted in a 1967 study of European immigrants to South Africa, where the prevalence of MS in immigrants was markedly higher than in the South African population. MS was also observed much more frequently in those who had been older than 15 when they migrated (Dean, 1967). The authors concluded that particular susceptibility to an environmental risk is set in childhood; the risk in those who migrated in childhood was offset by a protective exposure in South Africa.

Further studies have observed that people who migrate from countries with a high risk of MS to low-risk countries, or between high- and low-risk areas within the same country, have an MS risk intermediate between that of their place of origin and their new location (Gale and Martyn, 1995). MS prevalence in migrants who moved from the high-risk UK and Ireland to low-risk Australia was found to be significantly less than in their countries of origin. However, no difference was observed between the risk of MS in those migrating before or after 15 years of age, suggesting here that environmental risk of MS may accumulate over many years and is not specific to childhood and adolescence (Hammond et al., 2000).

Increased risk of MS in migrants moving from low- to high-risk countries is evidenced by the increasing prevalence of MS in individuals of Asian, African and Caribbean origin in the UK (Elian et al., 1990), and in a large proportion of MS patients of Middle-Eastern origin in Oslo (Smestad et al., 2008). The lack of age sensitivity and MS risk is reinforced in this latter study, many of whom had migrated to Norway beyond the age of 15. This finding suggests that environmental risk within the country of immigration extends into adulthood (Smestad et al., 2008). In a study of immigrants to the UK, children of migrants had an MS risk similar to that of other UK-born children

and higher than their parents, which adds further weight to the evidence of an environmental factor or factors implicated in MS aetiology (Elian et al., 1990).

Potential problems with migrant studies stem from the fact that people who migrate are not necessarily representative of the population of the country of origin. In ordinary circumstances migrants tend to be younger, healthier, and of a higher socioeconomic status than non-migrants from the same country (Gale and Martyn, 1995). Moreover, there may be differences in healthcare provisions between the country of origin and new country which can alter the probability of diagnosis. However, a study of return migration in the French West Indies found that individuals who had migrated to and lived in France for several years before returning to Martinique or Guadeloupe had more cases of MS than would be expected against the non-migrating population. Furthermore, the incidence was higher for those who had been living in France at the age of 15 or younger (Cabre et al., 2005). The authors suggest that as the background genetics of both migrating population and those who remained resident in the French West Indies were not dissimilar, an unfavourable environmental exposure may have been present to which those who were younger than 15 years were particularly susceptible. Therefore, there is little consensus on when and how the risk of MS changes, or age-sensitivity at time of migration. However, the literature remains consistent in terms of migration altering the risk of MS.

Further ecological evidence for an association between reduced sun exposure and MS risk has been accumulating. A month-of-birth effect was observed in studies of MS from the UK (Disanto et al., 2012; Willer et al., 2005), Scotland (Bayes et al., 2010), USA, Italy (Menni et al., 2012), Sicily (Salemi et al., 2000), Canada (Willer et al., 2005; Ramagopalan et al., 2009a; Sadovnick et al., 2007), Israel (Givon et al., 2012), Finland (Saastamoinen et al., 2012), and Scandinavia (Menni et al., 2012; Ramagopalan et al.,

2009a; Salzer et al., 2010). A systematic review and meta-analysis of these studies confirmed the effect; pregnancies that occur mainly over the spring and summer months deliver children with lower risk of MS (Dobson et al., 2012). The mechanism for this effect is hypothesised to be maternal vitamin D, supported through the interaction between magnitude of month of birth effect, and latitude as a proxy for ambient UV radiation levels. Maternal vitamin D should be at its peak during months of greater sunshine, and declining over winter months. Therefore, children in the Northern Hemisphere who are born in November are at less risk of MS than those born in May.

Animal models have also found UV exposure to selectively inhibit spinal cord demyelination (Wang et al., 2015), suppress EAE independent of vitamin D (Becklund et al., 2010), and reduce invariant natural killer T (iNKT) cell numbers (Yu and Cantorna, 2011). Additionally, a month-of-birth effect has been reproduced: overexpression of iNKT is protective against EAE, however low in-utero or neonatal vitamin D results in suboptimal numbers of iNKT. Moreover, early vitamin D deficiency appears to result in epigenetic changes to iNKT that cannot be reversed by subsequent vitamin D exposure (Yu and Cantorna, 2011), suggesting that in humans, prenatal vitamin D may be important in reducing future risk of autoimmunity.

### 6.1.5 Are these associations causal?

These ecological studies present evidence of an association between UV exposure, vitamin D, and MS risk, some of which has been reinforced in animal models of MS. However, establishing causation is a complex process for which Sir Austin Bradford Hill proposed a checklist for guidance (Hill, 1965). This checklist included criteria such as, among others, *strength* and *consistency* of associations, *temporality* – asserting that a cause must precede an effect, and biological *plausibility* for the way in

which a causal mechanism could result in the observed effect. However, Hill considered *experimental evidence* to provide the strongest support for causal inference (Hill, 1965).

As described above (section 6.1.4.1), observational studies cannot provide evidence that establishes causation. As well as chance, bias, and confounding which, while they can be reduced, cannot be entirely excluded, there is the issue of reverse causation. Reverse causation is particularly problematic for studies of vitamin D and UV exposure in illnesses with long latency periods such as MS. It is thus not clear from observational studies if low vitamin D levels result from poorer health and reduced exposure to UV because of increasingly lower levels of mobility, or if instead low vitamin D causes poorer health and reduced mobility.

There have been several systematic reviews of observational studies looking for associations between vitamin D and MS. A systematic review and meta-analysis of observational studies of vitamin D levels in people with MS found significantly lower vitamin D in cases compared to controls, and concluded that low vitamin D levels are associated with an increased risk of MS (Duan et al., 2014). However, two further systematic reviews used both observational and interventional study data. One of these studies explored vitamin D and autoimmune diseases, and found, like Duan et al. (2014), low vitamin D levels in people with MS compared to controls. They found no interventional studies of vitamin D supplementation and MS, however the observational studies that they included suggested that taking vitamin D supplements reduced risk of MS. They conclude that there is insufficient evidence to establish a causal link between circulating vitamin D levels and autoimmune disease risk, and highlight the need for more RCTs (Antico et al., 2012). The second systematic review found cross-sectional (but not longitudinal) observational evidence of an association between vitamin D and autoimmune disease, and within the included interventional literature, all of which were small trials, some promising results were observed on

reduced MS disease exacerbation when vitamin D supplements were supplied. They also conclude that existing evidence is weak, and call for longer term RCTs with higher vitamin D doses (Kriegel et al., 2011).

An umbrella review of observational systematic reviews and meta-analyses looked at environmental factors and MS. They found no association between vitamin D and MS, however there were consistent associations between MS and Epstein-Barr virus, smoking, and infectious mononucleosis (Belbasis et al., 2015). A further umbrella review that comprised observational systematic reviews and meta-analyses, and interventional meta-analyses, looked at the association between vitamin D and multiple health outcomes. They found no evidence of associations for autoimmune diseases. However, the review also highlighted a lack of meta-analyses for autoimmune outcomes, and again the need for more RCTs (Theodoratou et al., 2014).

### 6.1.6 Summary

A large body of work regarding vitamin D and MS has been produced, and has been summarised by multiple researchers. As described above, several systematic reviews of intervention and observational studies in the area of vitamin D and MS have been undertaken, and have been included in umbrella reviews. However, to date, whilst there is ample evidence to show an association between vitamin D and multiple sclerosis, there is a lack of evidence to indicate a causal relationship with the paucity of RCTs highlighted by multiple reviews.

The literature regarding MS, vitamin D, and UV exposure encompasses a large volume of observational, intervention, and genetic studies, with various methods used in different study designs. The disparate research that exists in this area lends itself to a scoping review, the methodology of which differs in salient ways from systematic reviews.

Systematic reviews, as discussed in section 6.1.4.3, require a focussed question to enable a rigorous evaluation of the literature, usually from a narrow range of quality-assessed studies. Scoping reviews, however, do not ask such narrow questions and neither do they assess the literature for quality. As such, the scoping review methodology only enables general observations of the literature regarding study designs and findings; the lack of quality assessment means that causal inference cannot be construed from a scoping review regardless of the methodologies of included studies. Instead, scoping reviews usually tackle broader research questions than systematic reviews, and aim to identify all literature relevant to the area of study. In so doing, the scoping review provides a method to "examine the extent, range, and research activity" (Arksey and O'Malley, 2005) within an area of interest. All relevant literature can then be mapped, findings aggregated, and results presented in a broad, narrative account. As well as the extent of research in key areas, gaps in the literature may be identified which could help to guide future research. Furthermore, when a series of apparently complementary findings are observed, the possibility for a future systematic review to assess the findings more closely may be highlighted.

However, despite their differences, both systematic reviews and scoping reviews should have methodologies that are rigorous and transparent (Arksey and O'Malley, 2005). The protocol (Appendix B) for the scoping review was designed according to the five-point framework of Arksey and O'Malley (Arksey and O'Malley, 2005), with reference to more recent research expanding on the scoping review methodology (Levac et al., 2010).

## 6.2 Methods

We searched Medline, Embase, Biosis, Web of Science, and the World Health Organisation (WHO) International Clinical Trials Registry Platform (ICTRP) for papers focussing on vitamin D or UV exposure in MS or EAE. We searched for grey literature

through the Proquest Dissertations and Theses Global database (PQDT), and Google Scholar. Searches were carried out on 21 March 2016.

Included articles defined MS according to the Poser (Poser et al., 1983) or McDonald (McDonald et al., 2001) criteria, or focussed on EAE as an animal model of MS. We included studies involving individual vitamin D and UV exposure measures, and MS patients of all ages. We included observational, experimental, and genetic studies. Ecological studies, and publications with no original data including meta-analyses and systematic reviews, were excluded. For reasons of time, papers in non-English languages were also excluded.

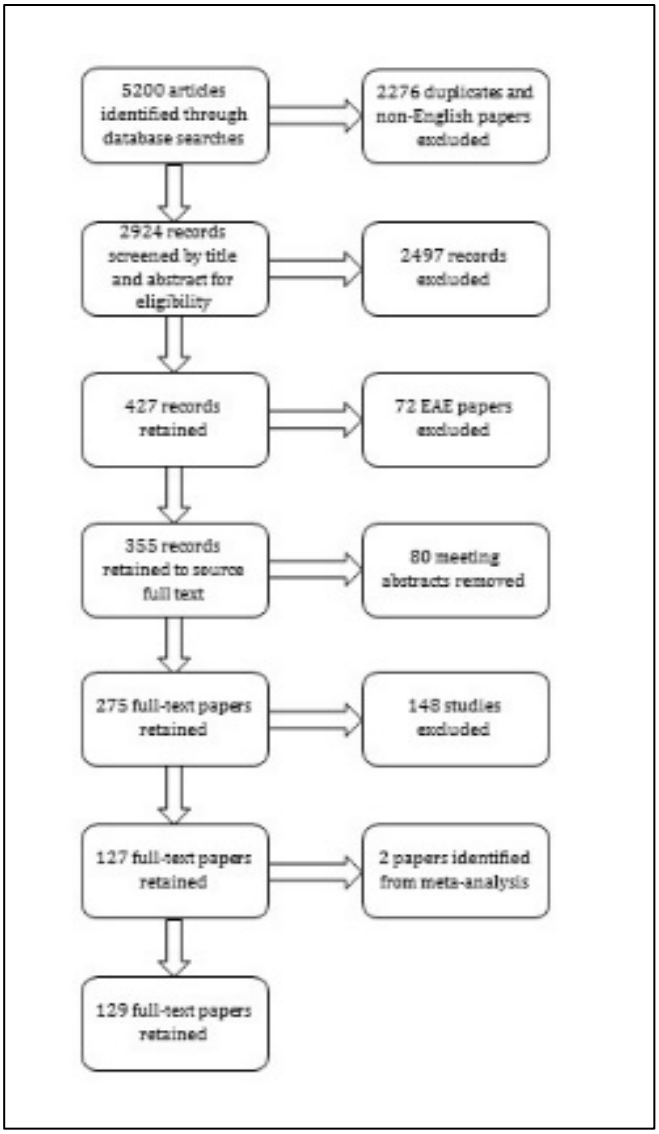
Search terms included, but were not limited to, vitamin D, cholecalciferol, ergocalciferol, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, ultra-violet radiation, sun exposure, multiple sclerosis, relapsing remitting multiple sclerosis, primary progressive multiple sclerosis and experimental autoimmune encephalomyelitis. We used boolean operators OR and AND to link search terms. The full search strategy is listed in the protocol (Appendix B).

Searches initially identified 5200 papers, of which 2219 were duplicates and 57 were foreign language papers. Because of the quantity of papers identified, we decided at this stage to exclude EAE and focus on vitamin D and UV in MS exclusively. We also decided to include all studies that involved MS patients regardless of diagnostic criteria, to understand how MS was being defined across studies. A total of 2924 potentially relevant papers were put through for eligibility screening. One person (EW) screened all titles and abstracts against eligibility criteria, whilst a second person (RM) screened a random sample of 10% for agreement. Both reviewers were in complete agreement about studies to be excluded. There was 75% agreement regarding papers to be retained for full screening; disagreements largely arose from the type of study and

publication under question, and were resolved through discussion. Two further papers were identified from a meta-analysis of genetic variants and MS risk.

In total, we extracted data from 129 papers which have been used in the main summary and report (Figure 6.1). Where possible, we also extracted and tabulated data from the meeting abstracts, although information from these was clearly less detailed and was not included in the main synthesis. Tables of abstract data are presented in Appendix C.

Figure 6.1 Flow diagram for paper selection



Exclusions from the title and abstract screening comprised studies which did not involve vitamin D or UV (651 excluded), or did not include MS patients (844 excluded). The other excluded papers were reviews, commentaries, chapters containing no new data, and case reports. Exclusions from the full-text screening were for similar reasons, usually where the title and abstract alone had included insufficient information to make a decision.

Papers fell largely into three categories of Observational, Intervention, and Genetic studies. Of the 127 identified papers, 72 were observational studies, 14 were intervention studies, and 43 were based on genetic analyses including the one Mendelian randomisation study.

The large number of papers meant that presenting the results in a meaningful way was challenging. We therefore present the results in three sections according to study type, ending each section with a summary of the main findings. The first section focusses on observational studies; the second on intervention studies, and the third on genetic studies. The Mendelian randomisation study is included at the end of the Genetic section, however it is discussed separately from the other genetic studies owing to the different type of question that this study asks.

As per Arksey and O'Malley's recommendations, we begin each results section with summary tables providing overviews of the study designs, the geographical distribution of studies, vitamin D and UV radiation measures, and MS criteria. This process identified themes within the literature which we go on to present in greater detail. A final discussion and conclusion brings together all the separate elements where we discuss the most striking themes from the literature.

### 6.2.1 Power and sample size

To provide context regarding the power of studies included in this review, I calculated the sample sizes required to detect several hypothetical effect sizes. To begin, I calculated the Cohen's d, or the standardised mean difference, in several studies included herein which had also been reported in Duan et al.'s systematic review (2011). The formula for Cohen's d is

$$d = \frac{m1 - m2}{\delta}$$

where m1 is the mean of group 1, m2 is the mean of group 2, and $\delta$ is the pooled standard deviation (Cohen, 1988).

The standardised effect sizes were -0.12 (Shahbeigi et al., 2013), -0.10 (van der Mei et al., 2007), and 0.11 (Lonergan et al., 2011). A Cohen's d of 0.2 reflects a small effect size, 0.5 is a medium effect size, and 0.8 is large (Cohen, 1988). As the standardised effect sizes obtained here suggest that the included studies identified small effects, I calculated the sample sizes required to detect small to medium effects, with power set at 0.80 and alpha at 0.05. The analyses showed that to detect an effect size of 1) d = 0.05 requires a sample size of 6280 in each group; 2) d = 0.1 requires 1570 in each group; 3) d = 0.2 requires 393 in each group, and 4) d = 0.5 requires a sample size of 64 participants in each group.

## 6.3 Results: Observational Studies

These results comprise studies where vitamin D or UV exposure had not been externally manipulated. Table 6.1 shows that most studies in this category were concerned solely with vitamin D levels in people with MS. The remainder focussed on UV exposure, or both UV exposure and vitamin D, in MS. Vitamin D measurement varied across studies. The most widely used was 25(OH)D. Nine studies estimated vitamin D from diet and supplements, either as a sole estimate of vitamin D, or as a contribution

to overall vitamin D including UV exposure estimates (Table 6.1). For UV, most studies relied on self-report measures of sun exposure which were usually retrospectively collected. Most of these studies used validated questionnaires, which were sometimes combined with a quantitative measure of long-term UV exposure such as actinic skin damage, or short term exposure such as polysulphone dosimeters (Table 6.1).

By a large margin the most frequent study design was case-control, followed by cross-sectional (Table 6.2). Prospective longitudinal and cohort studies, which are higher up the hierarchy of evidence, are more lengthy and expensive to conduct, and were fewer in number.

Table 6.1 Summary of measures and methods used for estimating vitamin D and UV exposure in full-text environmental studies (numbers do not add up as some studies collected data in multiple ways)

| | Outcome measures | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Aim** | **25(OH)D** | **$1,25(OH)_2D$** | **$24,25(OH)_2D_3$** | **DBP** | **Intake/ supplements** | **Self-report sun exposure** | **Quantitative sun exposure** | **No info/ other** |
| To assess blood vitamin D levels in patients with MS | 18 | 4 | - | 1 | - | - | - | - |
| To assess blood vitamin D and progression and severity of MS | 15 | 3 | 1 | - | - | - | - | - |
| To assess blood vitamin D and risk of MS onset | 10 | 1 | - | - | - | - | - | - |
| To assess the amount of lifetime UV exposure in MS | - | - | - | - | - | 2 | - | - |
| To assess childhood exposure to UV in MS | 1 | - | - | - | 1 | 5 | 1 | - |
| To assess UV exposure and mortality in MS | - | - | - | - | - | 2 studies estimated occupational sun exposure | | |
| To assess UV exposure and progression and severity of MS | - | - | - | - | 1 | 4 | - | 1 |
| To assess UV exposure and risk of MS onset | 1 | - | - | - | - | 5 | 1 | DBP SNPs |
| To assess vitamin D levels and lifetime UV exposure in MS | 3 | 1 | 1 | - | 2 | 5 | - | - |
| To assess vitamin D intake and risk of MS | - | - | - | - | 5 | 2 | - | - |

Table 6.2 Summary of full-text environmental studies by study design

| | Study design | | | | | | |
|---|---|---|---|---|---|---|---|
| **Aim** | **Case-control** | **Nested case-control** | **Prospective case-control** | **Cross-sectional** | **Prospective cohort** | **Retrospective cohort** | **No info/other** |
| To assess blood vitamin D levels in patients with MS | 14 | - | 1 | 2 | - | 1 | - |
| To assess blood vitamin D and progression and severity of MS | 4 | - | 2 | 3 | 5 | 1 | |
| To assess blood vitamin D and risk of MS onset | - | 3 | 2 | 1 | 2 | 1 | 1 (Prospective sub-study of clinical trial) |
| To assess the amount of lifetime UV exposure in MS | 2 | - | - | - | - | - | - |
| To assess childhood exposure to UV in MS | 3 | - | - | 2 | - | - | 1 (twin) |
| To assess UV exposure and mortality in MS | 1 | - | - | - | - | 1 | - |
| To assess UV exposure and progression and severity of MS | - | - | - | 4 | | | - |
| To assess UV exposure and risk of MS onset | 5 | - | - | - | - | - | - |
| To assess vitamin D levels and lifetime UV exposure in MS | 3 | - | - | 2 | | | |
| To assess vitamin D intake and risk of MS | 2 | - | - | - | 3 | - | - |

Definitions of MS also varied across studies. However, most studies referred to the McDonald criteria (any version thereof), which provides some consistency across studies. The older studies, before the introduction of the McDonald criteria, often used the Poser criteria. Only one study applied the Schumacher criteria, whilst others identified MS cases as those confirmed by MRI or neurologists without specifying the criteria against which diagnoses were made. The studies which contained no information about the identification, diagnosis, or confirmation of MS cases for inclusion, were more troubling (Table 6.3).

Geographically, studies were heavily weighted towards Europe and The Americas. The Eastern Mediterranean region was next best represented, with the contribution to this category largely from studies originating in Iran. South-East Asia was represented by two studies from India, and no identified studies originated in Africa. Five studies came from the Western Pacific region, including one from Japan and four from Australia (Table 6.4).

As anticipated, summarising the identified studies highlighted some gaps in research, particularly in terms of study design and geography. However, the process also enabled us to identify recurring themes which are discussed under separate headings below. Where study findings fall into multiple categories, repeated entries are made in the relevant tables.

Table 6.3 Summary of multiple sclerosis diagnostic criteria used in full-text environmental studies (numbers do not add up as some studies used multiple criteria to identify cases)

| | Criteria | | | | | |
|---|---|---|---|---|---|---|
| **Aim** | **Schumacher** | **Poser** | **McDonald** | **Neurologist confirmed/MRI** | **No info** | **Other** |
| To assess blood vitamin D levels in patients with MS | - | 4 | 15 | 2 | 1 | - |
| To assess blood vitamin D and progression and severity of MS | - | 4 | 11 | 2 | 2 | - |
| To assess blood vitamin D and risk of MS onset | - | 3 | 6 | 1 | - | - |
| To assess the amount of lifetime UV exposure in MS | - | 2 | - | - | - | - |
| To assess childhood exposure to UV in MS | 1 | 3 | 1 | 1 | 2 | - |
| To assess UV exposure and mortality in MS | - | - | - | - | - | 2 death certificates of MS death |
| To assess UV exposure and progression and severity of MS | - | 2 | - | - | 2 | - |
| To assess UV exposure and risk of MS onset | - | 1 | 4 | 1 | - | - |
| To assess vitamin D levels and lifetime UV exposure in MS | - | 3 | 1 | - | - | 1 self-report of dr diagnosis |
| To assess vitamin D intake and risk of MS | - | - | 2 | 3 | - | - |

Table 6.4 Summary of distribution of full-text environmental studies geographically (numbers do not add up as some studies cover multiple study regions)

| | Study region (according to WHO) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Aim** | **Africa** | **The Americas** | **South-East Asia** | **Europe** | **Eastern Mediterranean** | **Western Pacific** | **No info/ other** |
| To assess blood vitamin D levels in patients with MS | - | 6 | 1 | 5 | 5 | 2 | - |
| To assess blood vitamin D and progression and severity of MS | - | 5 | - | 8 | 3 | 1 | - |
| To assess blood vitamin D and risk of MS onset | - | 3 | - | 7 | - | - | - |
| To assess the amount of lifetime UV exposure in MS | - | 1 | - | - | 1 | - | - |
| To assess childhood exposure to UV in MS | - | 3 | - | 3 | - | 1 | - |
| To assess UV exposure and mortality in MS | - | 1 | - | 1 | - | - | - |
| To assess UV exposure and progression and severity of MS | - | 2 | - | 3 | - | - | - |
| To assess UV exposure and risk of MS onset | - | - | - | 2 | 2 | 1 | - |
| To assess vitamin D levels and lifetime UV exposure in MS | - | 1 | 1 | 1 | 1 | - | Web survey (57 Countries) |
| To assess vitamin D intake and risk of MS | - | 3 | - | 2 | - | - | - |

### 6.3.1 General associations between Vitamin D/UV exposure and MS (Table 6.5)

#### *Blood vitamin D in MS*

Twelve studies explored blood vitamin D levels in people with MS, with no specific focus on MS risk, onset, progression or subtype. All of these studies were case-control designs, and their findings were divided into two opposing groups. Eight found that people with MS had significantly lower blood vitamin D than controls (Karampoor et al., 2016; Shaygannejad et al., 2010; Fahmi et al., 2014; Gelfand et al., 2011; Kubicka and Pierzchała, 2013; Niino et al., 2015; Suresh Kumar et al., 2013; Pandit et al., 2013). The remaining four found that people with MS did not have significantly different blood vitamin D levels to controls (Lonergan et al., 2011; Moghtaderi et al., 2013; Eskandari et al., 2015; Barnes et al., 2007). Those results that showed significantly lower vitamin D came from studies from five of the six geographical regions, with sample sizes ranging from 700 cases and 1000 controls (Karampoor et al., 2016) to 25 cases and 25 controls (Fahmi et al., 2014), and 30 cases and 15 controls (Kubicka and Pierzchała, 2013). Sample sizes in the studies that found no significant differences were generally smaller, although the largest comprised 632 cases to 226 controls (Lonergan et al., 2011), and with geographical regions limited to Europe and the Eastern Mediterranean.

Blood vitamin D in CIS was explored in two longitudinal studies both from Europe. One was retrospective and the other prospective. Both studies found that patients with CIS had significantly lower vitamin D compared to controls, indicating that vitamin D deficiency is potentially implicated early in MS. However one study divided vitamin D into $25(OH)D_2$ and $25(OH)D_3$, and found that, although levels of $25(OH)D_3$ were significantly lower in people with CIS, levels of $25(OH)D_2$ did not differ between people with and without CIS (Behrens et al., 2016). These findings suggest that deficiency of $25(OH)D_3$, and not $25(OH)D_2$, may be an MS risk factor. Additionally, assessment of vitamin D bioavailability found no significant difference between people

with and without CIS, suggesting that the mechanism for vitamin D deficiency as an MS risk factor is not related to its bioavailability. Instead, the authors hypothesise, vitamin D may be a marker for a closely correlated and potentially causal mechanism (Behrens et al., 2016), such as UV exposure.

### *UV exposure in people with MS*

Two studies, with sample sizes of 1013 cases and 1194 controls (Bäärnhielm et al., 2012), and 195 cases and 146 controls (Al-Shammri et al., 2015), found that cases had significantly lower levels of UV exposure compared to controls. However, another study of 45 cases and 90 controls (Eskandari et al., 2015) found that there was no difference between groups regarding UV exposure.

Bäärnhielm et al.'s (2012) study originated in Europe, and utilised recall data of sun exposure for five years prior to study entry. Recall data may be influenced by response bias, which could strengthen the association between lack of UV and MS. The studies of Eskandari et al. (2015) and Al-Shammri et al. (2015), with their opposing findings, both originate from the Eastern Mediterranean; however Eskandari et al. (2014) (Iran) utilised measures of 25(OH)D as well as daily sun exposure whereas Al-Shammri et al. (2015) (Kuwait) relied solely on self-reported daily sun exposure. Additionally, although the two countries are within the same world region, they have different dress codes. The hijab is compulsory in Iran and not in Kuwait. The strict Iranian dress code may mean that UV exposure is dictated by clothing cover. Additionally, fully-shrouded dressing in Kuwait was more frequently observed in newly-diagnosed MS patients compared with established cases or controls (Al-Shammri et al., 2015).

### *UV exposure and MS mortality*

Two studies explored occupational UV exposure in relation to MS mortality. Both identified MS cases from death certificates, and relied on estimated measures of

occupational UV exposure depending upon the geographical area of occupation and the occupation itself. These studies were based in the USA (Freedman et al., 2000) and Sweden (Westberg et al., 2009), and used all MS deaths within their study regions giving sample sizes of 5701 cases and 839 cases respectively. Both studies found strong negative associations between occupational UV exposure and risk of death from MS. These results suggest that reduced UV exposure, and vitamin D, may be implicated in MS. However, reverse causation cannot be excluded, for example people with MS or subclinical disease may seek jobs that are sedentary and indoors.

Table 6.5 Studies exploring general associations between vitamin D or UV exposure and MS

| | Findings | Study design | Diagnostic criteria | Study region | Measure of vitamin D/ UV | Authors |
|---|---|---|---|---|---|---|
| **Blood vitamin D in MS** | Patients had significantly lower vitamin D than controls | Case-control | McDonald | Eastern Mediterranean | 25(OH)D | (Karampoor et al., 2016; Shaygannejad et al., 2010; Fahmi et al., 2014) |
| | | | | The Americas | | (Gelfand et al., 2011) |
| | | | | Europe | | (Kubicka and Pierzchata, 2013) |
| | | | | Western Pacific | $1,25(OH)_2D$ | (Niino et al., 2015) |
| | | | | South-East Asia | | (Suresh Kumar et al., 2013) |
| | | | | | 25(OH)D; sun exposure in childhood | (Pandit et al., 2013) |
| | Patients did not have significantly different vitamin D compared to controls | Case-control | McDonald | Europe | 25(OH)D | (Lonergan et al., 2011) |
| | | | | Eastern Mediterranean | | (Moghtaderi et al., 2013) |
| | | | | | 25(OH)D; daily sun-exposure question | (Eskandari et al., 2015) |
| | | | | Europe | $1,25(OH)_2D$ | (Barnes et al., 2007) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Patients with CIS had significantly lower vitamin D compared to controls | Prospective case-control | McDonald | Europe | 25(OH)$D_3$ | (Behrens et al., 2016) |
| | | Retrospective longitudinal study | Poser | Europe | 25(OH)D | (Martinelli et al., 2014) |
| | Patients with CIS did not have significantly lower vitamin D compared to controls | Prospective case-control | McDonald | Europe | 25(OH)$D_2$ | (Behrens et al., 2016) |
| **UV exposure in MS** | Childhood and adolescent sun exposure is associated with MS independent of vitamin D | Case-control | McDonald | South-East Asia | 25(OH)D; sun exposure in childhood | (Pandit et al., 2013) |
| | Cases had significantly lower UV exposure than controls | Case-control | McDonald | Eastern Mediterranean | Daily sun exposure and mode of dressing in structured interview | (Al-Shammri et al., 2015) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | Europe | Exposure to UVR in five years preceding entry to study (exposure to sunny weather, visits to sunny countries and use of sunbeds). Also 25(OH)D | (Bäärnhielm et al., 2012) |
| | Cases did not have significantly different UV exposure than controls | Case-control | McDonald | Eastern Mediterranean | 25(OH)D; daily sun-exposure question | (Eskandari et al., 2015) |
| **UV exposure and MS mortality** | Greater occupational UV exposure is associated with lower risk of MS mortality | Case-control | MS deaths | The Americas | Estimates of residential and occupational sun exposure | (Freedman et al., 2000) |
| | | Cohort | | Europe | Estimates of occupational sun exposure | (Westberg et al., 2009) |

### 6.3.2 Associations between vitamin D/UV exposure and MS risk and onset (Table 6.6)

#### *Blood vitamin D and risk of MS*

Four studies fell into the category of blood vitamin D and MS risk, all of which originated in Europe. The results were fairly consistent. Although one large nested case-control study found that high vitamin D in the years preceding disease onset was associated with decreased MS risk (Salzer et al., 2012), the other three studies, and a further result from the dissenting study, all found no association between vitamin D levels and MS risk. One continuous cross-sectional study included 164 optic neuritis (ON) patients who were prospectively followed to see if vitamin D levels in acute ON can predict later development of MS. While vitamin D levels were low in both groups, there was no significant difference in vitamin D between those who developed MS and those who did not (Pihl-Jensen and Frederiksen, 2015). This finding suggests that in cases of ON, vitamin D levels are not useful for predicting future MS development. A further prospective study of 525 incident MS cases and over 12,000 controls found that although vitamin D was inversely associated with the onset of any autoimmune disease, the association specifically with MS was not significant (Skaaby et al., 2015). Two studies, one nested case-control and one large prospective population-based case-control, were designed to investigate gestational (Salzer et al., 2012) and neonatal (Ueda et al., 2014) vitamin D respectively. Neither found evidence of an association between deficiency in these periods and later MS development.

#### *Blood vitamin D at MS onset*

Two case-control studies aimed to ascertain how blood vitamin D levels at MS diagnosis compared to controls without MS. One study, from the Eastern Mediterranean, comprised 75 cases and 100 controls who were relatives of cases (Mazdeh et al., 2013). The other study was from Europe and included 40 cases and 40 controls unrelated to cases (Soilu-Hänninen et al., 2005). Both studies found that

vitamin D levels were significantly lower in people with MS in the summer, but in no other seasons. Heat intolerance, which often affects people with MS (Emre, 1986), may account for this finding, as individuals with preclinical MS may instinctively avoid direct summer sunlight leading to reduced opportunity for vitamin D synthesis.

### *UV exposure in early life and MS risk*

Exposure to sunlight in early life and risk of MS was explored in six studies. One study of 1149 twin pairs specifically investigated childhood sun exposure (Islam et al., 2007). The others, all case-control with sample sizes ranging from 136 case and 272 controls to 1660 cases and 3050 controls, studied both childhood and adolescent sun exposure (Dalmay et al., 2010; Kampman et al., 2007; Bjornevik et al., 2014; van der Mei et al., 2003; Lucas et al., 2011). Studies originated from the Americas, Europe and Western Pacific regions, and all revealed significant associations between low sun exposure in early life and a greater risk of MS, revealing a similar pattern globally.

A further case-control study of over 100 cases and controls found that a lack of childhood UV exposure was a risk factor for MS, and that this association was independent of current vitamin D status (Pandit et al., 2013). This finding may support Behrens et al.'s (2016) above-mentioned hypothesis that vitamin D could simply be a marker for a correlated causal mechanism (Behrens et al., 2016). However, it may also reflect lower past vitamin D levels, which may be harmful to future autoimmunity.

### *UV exposure in early life and MS onset*

Two cross-sectional studies found that low sun exposure in both summer and winter during adolescence was significantly associated with a younger age of MS and RRMS onset (Laursen et al., 2016; McDowell et al., 2011a). These papers, with sample sizes of over 1000 and 500 cases respectively, suggest a mechanism whereby adolescent vitamin D deficiency, or a lack of UV, harms the development of the immune system. Again, this finding may complement those above studies which found a risk

period in childhood and adolescence for reduced UV exposure and MS risk; alternatively it may reflect lower past vitamin D levels. Both studies use self-reported measures of sun exposure. However, one study also used self-reported place of residence in childhood and adolescence to estimate UV exposure, which was weighted by the length of time spent in that location between ages 6 and 15, and self-reported use of sunscreen. The authors found a significantly younger age at onset in those who had regularly used sunscreen in childhood and early adolescence (McDowell et al., 2011a).

### *UV exposure and MS risk*

UV exposure and MS risk was explored more generally in four case-control studies. The sample sizes ranged from between 83 cases and 166 controls, to 1403 cases and 395 controls. All but one study agreed that reduced sun exposure over a lifetime is significantly associated with greater MS risk (Alonso et al., 2011; Mansouri et al., 2014; Lucas et al., 2011). This latter study (Lucas et al., 2011), from Australia, also found that vitamin D and reduced UV exposure may be independently associated with greater risk of a first demyelinating event, and so were consistent with there being separate roles for these two risk factors (Lucas et al., 2011). The findings from the fourth paper which had the smallest sample size of the four showed no evidence of an association between sun exposure and MS risk in the tropical climate of Mexico (Espinosa-Ramírez et al., 2014).

### *Vitamin D intake and MS risk*

Four studies were concerned with vitamin D intake and had similar findings. Two case-control studies found that low dietary vitamin D intake in childhood and adolescence (Kampman et al., 2007), or in adolescence only (Cortese et al., 2015), were associated with increased risk of MS. Cortese et al. (2015), in their sample of 935 cases and 1717 controls from Norway, also found that vitamin D intake in adolescence had a

protective effect although childhood intake did not. This finding suggests that deficiency or sufficiency in a specific period of life may present different risks for MS. Likewise, in a sample of 152 cases and 402 controls, cod liver oil consumption had a protective effect against MS in adolescents who reported low levels of outdoor activity during summer in Norway. This finding suggests that dietary supplementation can offset a lack of endogenous vitamin D and reduce MS risk (Kampman et al., 2007).

However, a prospective study of 379 incident and 67 prevalent cases in the USA found no association between adolescent vitamin D intake and MS risk (Munger et al., 2011). A further population-based case-control study of 1879 MS cases and 4135 controls from Sweden found that fatty fish consumption was associated with a decreased occurrence of MS. Dividing groups based on their reported consumption, the authors compared high (one to seven times per week) and low (monthly/seldom/never) intake, and found a significantly decreased occurrence of MS in the high intake group (Bäärnhielm et al., 2014). As no difference was observed between high and low intake of lean fish, the authors conclude that vitamin D, found in fatty fish, was a candidate for the protective effect.

### *Vitamin D intake and MS onset*

One cross-sectional study of 948 relapsing-remitting and secondary progressive MS cases was concerned with childhood and adolescent vitamin D intake, and MS onset. Cod liver oil intake between 6 and 15 years of age was associated with later onset age. This same study also found, as noted above, that low UV exposure and sunscreen use between 6 and 15 years was a risk for earlier MS onset (McDowell et al., 2011a). Although this study relies on self-reports of MS diagnosis and recall of childhood UV exposure and diet, the findings are nonetheless consistent with other literature, suggesting that childhood and adolescence may be an important period of

life in which to ensure adequate UV exposure or vitamin D sufficiency for future autoimmune health.

Table 6.6 Studies exploring vitamin D and UV in MS risk and MS onset

| | Findings | Study design | Diagnostic criteria | Study region | Measure of vitamin D/ UV | Authors |
|---|---|---|---|---|---|---|
| **Blood vitamin D and risk of MS** | Low vitamin D is associated with MS risk in the years preceding MS onset | Nested case-control | McDonald | Europe | 25(OH)D | (Salzer et al., 2012) |
| | Low vitamin D does not predict the conversion from optic neuritis to MS | Cross-sectional with some prospective follow-up | McDonald | Europe | 25(OH)D | (Pihl-Jensen and Frederiksen, 2015) |
| | Neonatal vitamin D is not associated with MS risk | Case-control | McDonald | Europe | 25(OH)D | (Ueda et al., 2014) |
| | Gestational vitamin D is not associated with MS risk | Nested case-control | McDonald | Europe | 25(OH)D | (Salzer et al., 2012) |
| | Low vitamin D is not significantly associated with MS onset | Prospective, population-based | McDonald | Europe | 25(OH)D | (Skaaby et al., 2015) |
| **Blood vitamin D at MS onset** | Patients had significantly lower vitamin D than controls (in summer) | Case-control | Poser/ McDonald | Europe | 25(OH)D | (Soilu-Hanninen et al., 2005) |
| | | | | Eastern Mediterranean | | (Mazdeh et al., 2013) |

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|---|---|---|---|---|---|---|
| **UV exposure in early life and MS risk** | Low sun exposure in childhood is significantly associated with increased risk of MS independent of vitamin D | Case-control | McDonald | South-east Asia | 25(OH)D; sun exposure in childhood | (Pandit et al., 2013) |
| | Low sun exposure in childhood is significantly associated with increased MS risk | Twin | Schumacher | The Americas | Comparative measures of self-and twin-reported sun exposure | (Islam et al., 2007) |
| | Low sun exposure in childhood and adolescence is significantly associated increased MS risk | Case-control | Poser | The Americas; Europe | Before the age of 15: sun exposure questionnaire; place and duration of residence and meteorological observations; travel abroad | (Dalmay et al., 2010) |
| | | | Poser or McDonald | Europe (North of Arctic Circle) | Ages 6-10, 11-15 and 16-20: sun exposure questionnaire; travel abroad; tanning/burning; sunbed use; cod-liver oil supplementation and often-eaten meals | (Kampman et al., 2007) |

| | | | | |
|---|---|---|---|---|
| | McDonald | Europe (multi-centre) | Sun exposure estimated from outdoor activity in winter and summer at different age periods; sunscreen use, skin, hair and eye colour and skin reaction to sun | (Bjornevik et al., 2014) |
| | MRI abnormalities and/or Poser | Western Pacific | Childhood and adolescent sun exposure questionnaire; vitamin D supplementation; actinic damage and skin phenotype by spectrophotometer | (Van Der Mei et al., 2003) |
| | Neurologist-confirmed | Western Pacific | Estimated sunlight exposure for different periods between ages 6 and present taking into account season, place of residence, and leisure time. Actinic damage measured and 25(OH)D | (Lucas et al., 2011) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **UV exposure in early life and MS onset** | Low sun exposure in adolescence is significantly associated with younger age at MS onset | Cross-sectional | Neurologist-confirmed (from treatment clinics) | Europe | 25(OH)D; adolescent sun exposure questionnaire | (Laursen et al., 2016) |
| | … and younger age at RRMS onset | | Self-report, followed by case-finding through statistical algorithm | The Americas | Childhood and adolescent sun exposure questionnaire; residential locations; vitamin D intake | (McDowell et al., 2011) |
| **Sunscreen use in early life and MS onset** | Sunscreen use in early life is associated with a younger age at RRMS onset | Cross-sectional | Self-report, followed by case-finding through statistical algorithm | The Americas | Childhood and adolescent sun exposure questionnaire; residential locations; vitamin D intake | (McDowell et al., 2011) |
| **UV exposure and MS risk** | There is no association between sun exposure and MS risk | Case-control | McDonald | The Americas | Phone interview of adolescent and recent sun exposure; travel | (Espinosa-Ramirez et al., 2014) |
| | Low lifetime sun exposure is significantly associated with an increased risk of MS | Case-control | McDonald | Eastern Mediterranean | Estimated sunlight exposure over lifetime prior to MS onset, in mins/day | (Alonso et al., 2011) |
| | | | Poser/McDonald | | Estimated sunlight exposure over lifetime prior to MS onset, hours/day | (Mansouri et al., 2014) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Neurologist-confirmed | Western Pacific | Estimated sunlight exposure for different periods between ages 6 and present taking into account season, place of residence, and leisure time. Actinic damage measured and 25(OH)D | (Lucas et al., 2011) |
| **Vitamin D intake and MS risk** | Higher adolescent vitamin D intake is significantly associated with lower MS risk | Case-control | McDonald | Europe | Childhood and adolescent cod liver oil or capsule use; frequency of supplement use; fatty fish intake; sun exposure estimated by summer outdoor activity | (Cortese et al., 2015) |
| | Higher childhood/adolescent vitamin D intake is significantly associated with lower MS risk | Case-control | Poser or McDonald | Europe (North of Arctic Circle) | Ages 6-10, 11-15 and 16-20: sun exposure questionnaire; travel abroad; tanning/burning; sunbed use; cod-liver oil supplementation and often-eaten meals | (Kampman et al., 2007) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Childhood vitamin D intake is not associated with MS risk | Case-control | McDonald | Europe | Childhood and adolescent cod liver oil or capsule use; frequency of supplement use; fatty fish intake; sun exposure estimated by summer outdoor activity | (Cortese et al., 2015) |
| | Adolescent vitamin D intake is not associated with MS risk | Prospective cohort study | Neurologist-confirmed | The Americas | High School FFQ; vitamin supplement use | (Munger et al., 2011) |
| | Fatty fish intake is significantly associated with decreased occurrence of MS | Case-control | McDonald | Europe | Dietary fatty fish intake; sun exposure in five years preceding study | (Baarnhielm et al., 2014) |
| **Vitamin D intake and MS onset** | Higher vitamin D intake in childhood/adolescence is associated with later onset of RRMS | Cross-sectional | Self-report, followed by case-finding through statistical algorithm | The Americas | Childhood and adolescent sun exposure questionnaire; residential locations; vitamin D intake | (McDowell et al., 2011) |

### 6.3.3 Associations between vitamin D/UV exposure and MS progression (Table 6.7)

#### *Blood vitamin D and MS progression*

The relationship between vitamin D levels and MS progression were explored in fourteen papers. Progression was variously measured by relapses, changes in disease activity on MRI, the Extended Disability Status Scale (EDSS), the Multiple Sclerosis Severity Score (MSSS), and Patient-Determined Disease Steps (PDDS). Two of these studies were prospective, with around 450 participants. Each study found that low blood vitamin D was significantly associated with increased risk of disease activity on MRI, relapses, and EDSS (Ascherio et al., 2014; Mowry et al., 2012a). A smaller prospective study also found that vitamin D levels were associated with disease activity in CIS (Mowry et al., 2016). Seven studies from Europe, The Americas, Eastern Mediterranean, and Western Pacific also found that low vitamin D was significantly associated with MS progression by MSSS or EDSS. Study designs were case-control and cross-sectional, with sample sizes ranging from 25 cases and 25 controls through to 469 cases. These complementary findings indicate a similar pattern globally, where low vitamin D is associated with greater MS progression (van der Mei et al., 2007; Fahmi et al., 2014; Weinstock-Guttman et al., 2011; Smolders et al., 2008; Mandia et al., 2014; Ascherio et al., 2014; Mowry et al., 2012b). A further two case-control studies (Shahbeigi et al., 2013; Kragt et al., 2009), each with around 100 cases, and 17 and 100 controls respectively, reported a similar association which was observed only in women. Although direction of causation could not be assessed, the findings complement those of the other studies, and suggest that low vitamin D may be more than a consequence of reduced activity.

Conversely, four case-control studies found that low vitamin D was not associated with MS progression by EDSS or MSSS. One of these studies comprised 339 cases and 342 controls, and focussed exclusively on African American participants

(Gelfand et al., 2011). Another was from Japan, with a relatively small sample size and low ratio of controls to cases (70 cases and 40 controls) (Niino et al., 2015). The remaining two studies were Iranian, one of which involved 700 cases and 1000 controls (Karampoor et al., 2016) and the other 168 cases and controls (Nikanfar et al., 2014). Although Fahmi et al.'s (2014) above-mentioned study with its opposing findings was also from Iran, it comprised a smaller sample size of 25 cases and 25 controls which may explain the differences in results.

### *UV exposure and MS progression*

UV exposure before MS onset and in MS progression was explored in six cross-sectional studies (Ramachandran et al., 2013; McDowell et al., 2011b; D'Hooghe et al., 2012; Mandia et al., 2014; Jelinek et al., 2015; Zivadinov et al., 2013). The findings were generally consistent. In three studies with sample sizes of 219 (McDowell et al., 2011b), 1372 (D'Hooghe et al., 2012) and 131 (Mandia et al., 2014) from the USA and Europe, low sun exposure was significantly associated with greater MS progression by MSSS, EDSS or PDSS. Intentional sun exposure was associated with 'mild' disability but was not related to relapse (Jelinek et al., 2015). Sun exposure was also positively associated with grey matter volume and whole brain volume independent of vitamin D in a cross-sectional analysis of 263 MS patients and 69 controls from the USA (Zivadinov et al., 2013).

Three cross-sectional studies explored sun-sensitive skin types and risk of MS severity measured by EDSS and MSSS, two of which were UK-based. One study found that fair skin and no history of childhood sun burning was associated with slow disease progression to EDSS stages 1-4 (Ramachandran et al., 2013); similarly the other found that sun-sensitive skin types had a reduced risk of MS severity by EDSS and MSSS in females only (Woolmore et al., 2007). Conversely, the third study from Belgium found that greater sun sensitivity was associated with greater disease progression to EDSS

stage 6 in PPMS (D'Hooghe et al., 2012). This latter study included 1372 respondents, and focussed exclusively on PPMS while the other two did not differentiate between MS subtypes, suggesting the possibility that progression of different subtypes is associated with different risks.

Finally, deliberate sun exposure recorded as a binary yes/no variable was not associated with health-related quality of life (HRQOL) as measured by the Multiple Sclerosis Quality of Life-54 questionnaire. Further findings from this study are discussed below (Jelinek et al., 2015).

### *Vitamin D intake and MS progression*

A significant positive association between vitamin D supplementation and relapse was found in one study (Jelinek et al., 2015), however after adjustment for other variables only latitude remained significantly predictive of relapse. With a sample size of 2469 people across 57 countries in Europe, The Americas, and Western Pacific, the study sample comprised self-reported doctor diagnosis of MS, vitamin D supplementation, quality of life, and deliberate sun exposure. Although these findings reinforce those of many of the above-mentioned studies, it is also limited in several ways. Specifically, relying on non-confirmed self-reports of MS diagnosis may mean that the sample contains people who suspect they have MS but have not been through a formal diagnostic procedure. Additionally, the self-report measures would be subject to recall bias, which is particularly problematic if the people within the study have awareness of the potential importance of these risk factors to their illness.

Table 6.7 Studies exploring associations between vitamin D/UV and MS progression

| | Findings | Study design | Diagnostic criteria | Study region | Measure of vitamin D/ UV | Authors |
|---|---|---|---|---|---|---|
| **Blood vitamin D and MS progression** | Low vitamin D is significantly associated with increased risk of disease activity | Prospective cohort study | Poser/McDonald | Europe; The Americas | 25(OH)D | (Ascherio et al., 2014) |
| | | | McDonald | The Americas | | (Mowry et al., 2012) |
| | … and is associated with activity in CIS | Prospective sub-study of clinical trial | MRI | The Americas | | (Mowry et al., 2016) |
| | Low vitamin D is significantly associated with MS progression by EDSS or MSSS | Case-control | Poser/MRI | Western Pacific | 25(OH)D | (Van Der Mei et al., 2007) |
| | | | McDonald | Eastern Mediterranean | | (Fahmi et al., 2014) |
| | | Cross-sectional | | The Americas | | (Weinstock-Guttman et al., 2011) |
| | | | | Europe | | (Smolders et al., 2008) |
| | | | | | Dietary questionnaire and sunlight exposure for two years preceeding study; 25(OH)D | (Mandia et al., 2014) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Low vitamin D is significantly associated with MS progression by EDSS or MSSS in women only | Case-control | McDonald | Eastern Mediterranean | 25(OH)D | (Shahbeigi et al., 2013) |
| | | Prospective case-control | Poser | Europe | 25(OH)D; $1,25(OH)_2D$ | (Kragt et al., 2009) |
| | Low vitamin D is not significantly associated with MS progression by EDSS or MSSS | Case-control | McDonald | Eastern Mediterranean | 25(OH)D | (Karampoor et al., 2016; Nikanfar et al., 2014) |
| | | | | Western Pacific | | (Niino et al., 2015) |
| | | | | The Americas | $1,25(OH)_2D$ | (Gelfand et al., 2011) |
| **UV exposure MS progression** | Low sun exposure in autumn/winter before disease onset is significantly associated with increased risk of progression to PDDS score 8 | Cross-sectional | Self-report, followed by case-finding through statistical algorithm | The Americas | Childhood and adolescence self-report sun exposure; dietary and supplement intake from age 6 to symptom onset | (McDowell et al., 2011b) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Lower levels of sun exposure in 10 years preceding study was significantly associated with an increased risk of progressing to EDSS 6 in RRMS | Cross-sectional | "Definite diagnosis" | Europe | Sun exposure questionnaire; skin type; lifetime burning | (D'Hooghe et al., 2012) |
| | Low sun exposure is significantly associated with higher MSSS | Cross-sectional | McDonald | Europe | Dietary questionnaire and sunlight exposure for two years preceeding study; 25(OH)D | (Mandia et al., 2014) |
| | Intentional sun exposure is significantly associated with being in the mild disability group but is not related to relapse | Cross-sectional | Self-report of doctor diagnosis | Web-based survey of 57 countries inc. Europe, The Americas and Western Pacific | Frequency and dosage of vitamin D supplementation; deliberate sun exposure | (Jelinek et al., 2015) |
| | High summer sun exposure is significantly associated with increased grey matter volume and whole brain volume independent of vitamin D | Case-control | Neurological and MRI examination | The Americas | $25(OH)D_3$; $1,25(OH)_2D_3$; $24,25(OH)_2D_3$; supplement use, skin type and sun exposure questionnaires | (Zivadinov et al., 2013) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Increased sun sensitivity is associated with an increased risk of progression to EDSS 6 in PPMS | Cross-sectional | Definite diagnosis" | Europe | Sun exposure questionnaire; skin type; lifetime burning | (D'Hooghe et al., 2012) |
| | Fitzpatrick skin phenotypes 1 and 2, and no history of childhood sun burning is associated with slow MS progression by EDSS 1-4 | Cross-sectional | Poser | Europe | Self-administered sun-exposure questionnaire and childhood sunburning; skin phenotype | (Ramachandran et al., 2013) |
| | Sun-sensitive skin types have reduced risk of MS severity by EDSS and MSSS in females | Cross-sectional | Poser | Europe | Skin type questionnaire; childhood, adolescent and adulthood sun exposure and sun burning | (Woolmore et al., 2007) |
| | Deliberate sun exposure is not related to HRQOL after adjustment for confounders | Cross-sectional | Self-report of doctor diagnosis | Web-based survey of 57 countries inc. Europe, The Americas and Western Pacific | Frequency and dosage of vitamin D supplementation; deliberate sun exposure | (Jelinek et al., 2015) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Vitamin D intake and MS progression** | Vitamin D supplementation is significantly associated with relapse only before adjustment | Cross-sectional | Self-report of doctor diagnosis | Web-based survey of 57 countries inc. Europe, The Americas and Western Pacific | Frequency and dosage of vitamin D supplementation; deliberate sun exposure | (Jelinek et al., 2015) |

### 6.3.4 Associations between vitamin D/UV exposure and MS subtype (Table 6.8)

#### *Blood vitamin D and MS subtype*

Fourteen papers explored blood vitamin D and MS subtype, including progressive disease and RRMS. Two of these studies, one cross-sectional with 267 cases (Smolders et al., 2008), and the other a retrospective cohort study of 181 cases (Thouvenot et al., 2015), found that vitamin D levels were significantly lower in patients with progressive MS compared to RRMS. These studies were both European, however another cross-sectional study from The Americas explored vitamin D levels in adult-onset MS (59 cases), young-adult-onset MS (33 cases) and childhood-onset MS (24 cases). No differences in vitamin D levels were observed between these three groups, suggesting that vitamin D deficiency may be irrelevant to onset age. Instead, factors including race and obesity were significantly associated with low vitamin D across all groups (Brenton et al., 2014).

Three case-control studies found that patients with RRMS had significantly lower vitamin D levels than controls. It is notable, however, that one of these studies had a small sample size of 29 cases and 29 controls (Polachini et al., 2016), and the other two had a different numbers of cases and controls, with 92 cases to 60 controls (Correale et al., 2011) and 98 cases to 17 controls (Shahbeigi et al., 2013). Two of these studies were European and the latter Eastern Mediterranean.

However, results from two further case-control studies, both from the Eastern Mediterranean, found that, in contrast, vitamin D levels were not significantly different between people with RRMS and controls. Both these studies involved equal numbers of cases and controls, with 37 of each in one study (Hejazi et al., 2014) and 168 of each in the other (Nikanfar et al., 2014).

Eight studies had findings relating to vitamin D levels and relapse in RRMS. Five of these were case-control studies (Soilu-Hänninen et al., 2005; Correale et al., 2011; Pandit et al., 2013; Mazdeh et al., 2013; Nikanfar et al., 2014), with sample sizes ranging from 40 cases and 40 controls, to 168 cases and 168 controls. Findings were consistent across all studies, showing that patients in periods of relapse had significantly lower vitamin D than those in remission. Similarly, the other three studies found that patients with higher vitamin D levels were less likely to experience relapse. One of these studies was cross-sectional (Smolders et al., 2008), and the other two were prospective (Simpson et al., 2010; Runia et al., 2012), with sample sizes of 267, 145 and 73 respectively. Only one study, with 40 PPMS cases and 60 controls, had a finding relating to vitamin D in PPMS. Unlike the RRMS results, no difference in vitamin D levels was observed between people with PPMS and controls (Correale et al., 2011).

### *UV exposure and MS subtype*

One study explored UV exposure and progression in RRMS and PPMS leading to two findings. We discussed this study above under the UV exposure and MS progression section in Table 6.7 but, because the results were subtype-specific, we also present them in Table 6.8 for the sake of clarity (D'Hooghe et al., 2012).

### *Vitamin D in paediatric-onset MS*

Two longitudinal studies explored vitamin D in paediatric-onset MS. One was a prospective study of 302 children with acute demyelinating syndrome, 63 of which were diagnosed with MS. The results showed that low vitamin D was significantly associated with MS onset in children (Banwell et al., 2011), a finding at odds with Brenton et al.'s (2014) above-mentioned study. The other study of 100 paediatric MS cases was retrospective, and found that low vitamin D was significantly associated with relapse rate in childhood MS and CIS (Mowry et al., 2010).

Table 6.8 Studies exploring vitamin D/UV exposure and MS subtype

| | Findings | Study design | Diagnostic criteria | Study region | Measure of vitamin D/ UV | Authors |
|---|---|---|---|---|---|---|
| **Blood vitamin D and MS subtype** | Patients with progressive disease have significantly lower vitamin D compared to RRMS patients | Cross-sectional | McDonald | Europe | 25(OH)D | (Smolders et al., 2008) |
| | | Retrospective cohort study | | | | (Thouvenot et al., 2015) |
| | There is no difference in vitamin D between MS subtypes | Cross-sectional | McDonald | The Americas | 25(OH)D | (Brenton et al., 2014) |
| | Patients with RRMS had significantly lower vitamin D than controls | Case-control | McDonald | The Americas | 25(OH)D | (Polachini et al., 2016; Correale et al., 2011) |
| | | | | Eastern Mediterranean | | (Shahbeigi et al., 2013) |
| | Patients with RRMS did not have significantly different vitamin D to controls | Case-control | McDonald | Eastern Mediterranean | 25(OH)D | (Hejazi et al., 2014; Nikanfar et al., 2014) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Patients with RRMS in relapse had significantly lower vitamin D than patients in remission | Case-control | Poser/McDonald | Europe | 25(OH)D | (Soilu-Hanninen et al., 2005) |
| | | | | The Americas | | (Correale et al., 2011) |
| | | | McDonald | South-East Asia | 25(OH)D; sun exposure in childhood | (Pandit et al., 2013) |
| | | | Poser/McDonald | Eastern Mediterranean | 25(OH)D | (Mazdeh et al., 2013) |
| | | | McDonald | | | (Nikanfar et al., 2014) |
| | Patients with high vitamin D levels were more likely to avoid relapse in RRMS | Cross-sectional | McDonald | Europe | 25(OH)D | (Smolders et al., 2008) |
| | | Prospective cohort study | | Western Pacific | | (Simpson et al., 2010) |
| | | | | Europe | | (Runia et al., 2012) |
| | Patients with PPMS did not have significantly different vitamin D compared to controls | Case-control | Poser/McDonald | The Americas | 25(OH)D | (Correale et al., 2011) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **UV exposure and MS subtype** | Lower levels of sun exposure in 10 years preceding study was significantly associated with an increased risk of progressing to EDSS 6 in RRMS | Cross-sectional | "Definite diagnosis" | Europe | Sun exposure questionnaire; skin type; lifetime burning | (D'Hooghe et al., 2012) |
| | Increased sun sensitivity is associated with an increased risk of progression to EDSS 6 in PPMS | Cross-sectional | "Definite diagnosis" | Europe | Sun exposure questionnaire; skin type; lifetime burning | (D'Hooghe et al., 2012) |
| **Vitamin D in paediatric-onset MS** | Low vitamin D is significantly associated with risk of paediatric-onset MS | Prospective national cohort | McDonald | The Americas | 25(OH)D | (Banwell et al., 2011) |
| | Low vitamin D is significantly associated with relapse rate in paediatric-onset MS or CIS | Longitudinal cohort study | Neurologist-confirmed | The Americas | 25(OH)D | (Mowry et al., 2010) |

### 6.3.5 Associations between vitamin D/UV exposure and sex in MS (Table 6.9)

#### *Blood vitamin D in MS and sex*

Six of our studies had sex-specific findings relating to blood vitamin D in MS, two of which showed an association between low vitamin D and greater MS progression in women and were discussed above in Table 6.7 (Shahbeigi et al., 2013; Kragt et al., 2009). A cross-sectional study from a tertiary care hospital in the USA showed extensive vitamin D insufficiency in the 80 female patients (Nieves et al., 1994). One case-control study of 30 cases and 15 controls in Poland found that vitamin D levels were also lower in women compared with men at a similar stage of disease (Kubicka and Pierzchała, 2013). Another prospective case-control study in the Netherlands, with 101 cases and 107 controls, found that low vitamin D levels were associated with increased odds of MS in women (Kragt et al., 2009).

Two studies, however, found positive associations between MS and vitamin D in women. One case-control study from the UK found that women had significantly higher vitamin D compared to men (Barnes et al., 2007), in direct contrast to Kubicka and Pierzchala (2013). The other study from Romania found that higher vitamin D was associated with better clinical outcomes in women, however this finding was not significant (Kinga and Balasa, 2015). Both studies were relatively small, with 29 cases and 22 controls, and 36 cases respectively.

#### *UV exposure in MS and sex*

Two cross-sectional studies had sex-specific findings regarding UV exposure, one of which showed an association between greater sun-sensitivity and reduced risk of severe disease in females, and was discussed above in Table 6.7 (Woolmore et al., 2007). The second study found that almost half of the 80 women with MS comprising the study population reported no weekly sun exposure (Nieves et al., 1994).

### *Vitamin D intake in MS and sex*

Two studies with female-only samples explored vitamin D intake in MS. One cross-sectional study found that vitamin D intake was much lower than the recommended level (Nieves et al., 1994). The other prospective cohort study had two relevant findings. Firstly, vitamin D from food had no association with MS incidence; however vitamin D intake from supplements was significantly associated with a reduced risk of MS (Munger et al., 2004).

Table 6.9 Studies exploring the association between vitamin D/UV in MS and sex

| | Findings | Study design | Diagnostic criteria | Study region | Measure of vit D/UV | Authors |
|---|---|---|---|---|---|---|
| **Blood vitamin D in MS and sex** | Mean vitamin D of female patients with MS was in the insufficiency range | Cross-sectional | Neurologist-confirmed | The Americas | 25(OH)D | (Nieves et al., 1994) |
| | Women with MS had significantly higher vitamin D than men with MS | Case-control | No information (recruited from MS support groups) | Europe | 25(OH)D; $1,25(OH)_2D$ | (Barnes et al., 2007) |
| | Low vitamin D is significantly associated with increased odds of MS in women only | Prospective case-control | Poser | Europe | 25(OH)D; $1,25(OH)_2D$ | (Kragt et al., 2009) |
| | Low vitamin D is significantly associated with MS progression by EDSS or MSSS in women only | Case-control | McDonald | Eastern Mediterranean | 25(OH)D | (Shahbeigi et al., 2013) |
| | | Prospective case-control | Poser | Europe | 25(OH)D; $1,25(OH)_2D$ | (Kragt et al., 2009) |
| | Vitamin D is significantly lower in women compared to men at an advanced disease stage with similar levels of physical disability | Case-control | McDonald | Europe | 25(OH)D | (Kubicka and Pierzchata, 2013) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Higher vitamin D is associated (insignificantly) with better clinical outcomes in female patients | Cross-sectional | Neurologist-confirmed | Europe | 25(OH)D | (Kinga and Balasa, 2015) |
| **UV exposure in MS and sex** | 40% of female patients with MS report no weekly sun exposure | Cross-sectional | Neurologist-confirmed | The Americas | 25(OH)D | (Nieves et al., 1994) |
| | Sun-sensitive skin types have reduced risk of MS severity by EDSS and MSSS in females | Cross-sectional | Poser | Europe | Skin type questionnaire; childhood, adolescent and adulthood sun exposure and sun burning | (Woolmore et al., 2007) |
| **Vitamin D intake in MS and sex** | Vitamin D intake in 80% of female patients with MS is below the recommended level | Cross-sectional | Neurologist-confirmed | The Americas | 25(OH)D | (Nieves et al., 1994) |
| | Vitamin D intake from food is not associated with MS incidence in women | Prospective cohort study | Neurologist-confirmed | The Americas | Semiquantitative FFQ | (Munger et al., 2004) |
| | Vitamin D intake from supplements is significantly associated with reduced risk of MS in women | | | | | (Munger et al., 2004) |

### 6.3.6 Summary

We identified a large number of observational studies regarding vitamin D and UV exposure in MS, including general associations of these risk factors in people with MS, associations with MS risk, onset and progression, associations with different MS subtypes and associations with sex. The literature was large and heterogeneous, however overall, the following themes emerged:

1. **General associations:** case-control studies from many different countries found lower vitamin D, and lower UV exposure, in cases compared to controls. Low vitamin D was also found longitudinally in patients with CIS. Where no difference was observed between cases and controls the studies tended to be smaller, suggesting a lack of power to detect an effect, or from world regions with specific customs which may create contrasting results.
2. **MS risk and onset:** multiple case-control, prospective and cross-sectional studies across several regions globally investigated vitamin D levels and MS risk and onset. Those studies that specifically investigated vitamin D deficiency and risk of MS, and found no association between vitamin D levels and MS risk. However, in those studies that measured vitamin D at MS diagnosis, lower vitamin D levels were more often present in MS at the time of diagnosis compared to controls. Twin, case-control and cross-sectional studies from three world regions found that reduced sun exposure in early life was associated with a greater risk of MS or younger onset age. The case-control studies exploring early life vitamin D intake and MS risk had conflicting results, however a prospective cohort study concurred with one of the studies, finding no association with MS risk.
3. **MS progression:** fourteen prospective, case-control and cross-sectional studies from five world regions found that low vitamin D was associated with greater

disease activity. The five papers that dissented from this finding involved world regions with specific customs, or certain populations such as African Americans, which may have a local effect on vitamin D levels. Associations between UV exposure and MS were mostly explored in cross-sectional studies which generally agreed that greater sun exposure was associated with slower disease progression, and was positively associated with brain volume independent of vitamin D status. Inconsistencies were observed regarding skin sensitivity and MS.

4. **MS subtype:** studies from five world regions found that vitamin D was significantly lower in people with RRMS than controls, and in patients in relapse compared to remission. Additionally, people with higher levels of vitamin D were less likely to experience relapse. However, no association was observed with PPMS although only one study explored this. Two cohort studies, one prospective and one retrospective, found that low vitamin D was associated with paediatric onset MS or paediatric relapse rate.
5. **Sex:** five of the eight studies in this section found significant associations between low vitamin D and MS, including risk and progression, in women. A lack of sun exposure and vitamin D intake in women with MS was also observed, although a prospective cohort study found that supplement use was protective. These studies largely originated from Europe and The Americas.

Observational studies therefore show associations between MS, low vitamin D levels, and UV exposure. However, these observational associations require exploration in the interventional literature to see if observed effects can be replicated under controlled conditions.

## 6.4 Results: Intervention Studies

These results comprise studies involving an externally manipulated intervention. The intervention studies comprise double-blind RCTs and double-blind, placebo-controlled RCTs (Table 6.10). A few other study designs were used including two open-label trials and three others which were less conventional, and did not fit a defined category.

All intervention papers focussed on vitamin D supplementation as the exposure (Table 6.11). Various types of vitamin D were used, although most widely investigated was $25(OH)D_3$. However, $25(OH)D_2$, 25(OH)D, $1,25(OH)_2D$ and alfacalcidol, a vitamin D analogue, were also explored under controlled conditions.

The McDonald criteria was often used to identify cases (Table 6.12), although one older study had used the Schumacher criteria. There was only one paper which failed to identify how their sample of MS patients had been diagnosed. One study, which concentrated on conversion from CIS to MS identified their sample as those who had not yet converted to MS by the McDonald criteria.

Geographically, studies arose most often from The Americas and Europe (Table 6.13). No studies originated from Africa or South-East Asia. The Eastern Mediterranean region was represented solely by papers from Iran, and the Western Pacific region by Australia.

Table 6.10 Summary of full-text experimental study designs

| | Study design | | | | |
|---|---|---|---|---|---|
| **Aims** | **Double-blind RCT/pilot RCT** | **Double-blind, placebo-controlled RCT** | **Open-label study** | **Open-label pilot study** | **Other** |
| To establish the safety and/or tolerability of vitamin D in MS | 1 | - | - | 1 | - |
| To assess blood vitamin D levels in MS following vitamin D therapy | 1 | 2 | - | - | 1 (retrospective evaluation) |
| To assess whether vitamin D can prevent or delay conversion to CDMS | - | 1 | - | - | - |
| To assess the effect of vitamin D on disease progression by EDSS | 1 | 2 | - | - | - |
| To assess the effect of vitamin D on disease progression by relapse | 1 | 2 | 1 | - | 1 (observational, uncontrolled study)<br>1 (experimental) |
| To assess the effect of vitamin D on disease progression by MRI activity | 1 | 2 | - | - | |

Table 6.11 Summary of interventions in full-text experimental studies (vitamin D/UV only)

| | Interventions | | | | |
|---|---|---|---|---|---|
| **Aim** | **25(OH)D** | **25(OH)$D_2$** | **25(OH)$D_3$** | **1,25(OH)$_2$D** | **Alfacalcidol** |
| To establish the safety and/or tolerability of vitamin D in MS | - | - | 1 | 1 | - |
| To assess blood vitamin D levels in MS following vitamin D therapy | 1 | 1 | 2 | - | - |
| To assess whether vitamin D can prevent or delay conversion to CDMS | - | - | 1 | - | - |
| To assess the effect of vitamin D on disease progression by EDSS | - | 1 | 2 | - | - |
| To assess the effect of vitamin D on disease progression by relapse | - | - | 5 | - | 1 |
| To assess the effect of vitamin D on disease progression by MRI activity | - | 1 | 2 | - | - |

Table 6.12 Summary of multiple sclerosis diagnostic criteria used in full-text experimental studies

| | Criteria | | | | | |
|---|---|---|---|---|---|---|
| **Aim** | **Schumacher** | **Poser** | **McDonald** | **Neurologist confirmed/MRI** | **No info** | **Other** |
| To establish the safety and/or tolerability of vitamin D in MS | - | - | 1 | - | 1 | - |
| To assess blood vitamin D levels in MS following vitamin D therapy | - | - | 4 | - | - | - |
| To assess whether vitamin D can prevent or delay conversion to CDMS | | | | | | Non-fulfilment of McDonald 2005 criteria |
| To assess the effect of vitamin D on disease progression by EDSS | - | - | 3 | - | - | - |
| To assess the effect of vitamin D on disease progression by relapse | 1 | - | 5 | - | - | - |
| To assess the effect of vitamin D on disease progression by MRI activity | - | - | 3 | - | - | - |

Table 6.13 Summary of distribution of full-text experimental studies geographically

| | Study region (according to WHO) | | | | | |
|---|---|---|---|---|---|---|
| **Aim** | **Africa** | **The Americas** | **South-East Asia** | **Europe** | **Eastern Mediterranean** | **Western Pacific** |
| To establish the safety and/or tolerability of vitamin D in MS | - | 2 | - | - | - | - |
| To assess blood vitamin D levels in MS following vitamin D therapy | | 1 | - | 2 | - | 1 |
| To assess whether vitamin D can prevent or delay conversion to CDMS | - | - | - | - | 1 | - |
| To assess the effect of vitamin D on disease progression by EDSS | | - | - | 1 | 1 | 1 |
| To assess the effect of vitamin D on disease progression by relapse | - | 2 | - | 4 | - | - |
| To assess the effect of vitamin D on disease progression by MRI activity | - | - | - | 1 | 1 | 1 |

### 6.4.1 Safety and tolerability of vitamin D therapy in MS (Table 6.14)

Establishing the safety and tolerability of a potential new treatment is vital in drug development to ensure that any resulting therapy is safe with minimal side-effects. Two papers from North America, one double-blind RCT (Wingerchuk et al., 2005) and one open-label pilot study (Sotirchos et al., 2016), explored the safety and tolerability of vitamin D therapy in people with MS. Both found that vitamin D is safe and well-tolerated (Wingerchuk et al., 2005; Sotirchos et al., 2016). These two trials had sample sizes of 15 RRMS patients (Wingerchuk et al., 2005), and 40 RRMS patients in two groups of 20 (Sotirchos et al., 2016). The amount of vitamin D administered varied substantially between the two trials from 100 IU/day to 10,400 IU/day, and furthermore the intervention duration varied from 48 weeks to 26 weeks respectively. The effect of higher doses over longer periods is therefore not well understood from these studies.

### 6.4.2 Blood vitamin D levels following vitamin D therapy in MS (Table 6.14)

Four studies evaluated vitamin D levels in people with MS following vitamin D therapy, either as the primary research question or as part of a wider study. One double-blind RCT from Australia with 23 participants (Stein et al., 2011), and one retrospective evaluation of 49 US patients who had been taking high-dose versus low-dose vitamin D for at least three months (Hiremath et al., 2009), both found that those taking a high dose had significantly higher vitamin D. Additionally, a double-blind placebo-controlled RCT from Norway involving 36 treatment and 32 placebo participants, found that those in the treatment group had significantly higher vitamin D levels (Røsjø et al., 2015). A further study involving the same dataset as Røsjø et al. (2015) found that people in the treatment group had sufficient year-round vitamin D, however those in the placebo group had generally low vitamin D levels which increased only in the summer months (Steffensen et al., 2013). Only one study administered

25(OH)$D_2$, and the rest used 25(OH)$D_3$. Intervention times ranged from 'at least' 13 weeks, to 96 weeks. However, these studies are all in agreement that supplementing with vitamin D, and with higher doses of vitamin D, is effective at raising blood vitamin D levels in people with MS.

### 6.4.3 Vitamin D therapy and disease progression (Table 6.14)

Having determined if a treatment is safe and has a biological effect, it is then important to assess its effectiveness on disease outcomes. The remaining studies in this section focus on whether trials of vitamin D supplementation showed any effect on MS onset or MS progression.

#### *6.4.3.1 Vitamin D therapy and conversion to clinically definite MS*

One study explored whether vitamin D therapy could prevent or delay conversion from ON to clinically definite MS (CDMS) (Derakhshandi et al., 2013). This double-blind, randomised, parallel-group trial was conducted in Iran. Patients with confirmed ON who did not fulfil McDonald's MS criteria were randomly assigned to receive vitamin D or a placebo. During the twelve-month follow-up period, five of the eleven participants in the placebo group experienced a second demyelinating attack, compared to none of the thirteen treatment group participants. Although promising, small numbers, and a short follow-up period mean these results should be interpreted cautiously, however they may warrant a larger clinical trial.

#### *6.4.3.2 Vitamin D therapy and disease progression by EDSS*

Three studies explored the impact of vitamin D therapy on the progression of MS assessed by the EDSS. Two RCTs that administered vitamin $D_3$ in the treatment group versus a placebo group found no differences in disease progression when scored by EDSS (Kampman et al., 2012; Mosayebi et al., 2011). These studies were conducted in Norway and Iran respectively, ran for 96 and 26 weeks, and varied between daily and monthly vitamin D supplementation. However, one further study from Australia, of

vitamin $D_2$ administered in a high dosage to one treatment group and low dosage to another, found that those who were given the high dosage had better EDSS scores (Stein et al., 2011). This study had a smaller sample size compared to the other two studies, did not include a placebo group, ran for 26 weeks, and involved daily supplementation. The methodological differences of this study compared with the others may account for some of the differences observed, however possibly of most interest is the difference in findings between the study exploring $25(OH)D_2$ and those looking at $25(OH)D_3$.

#### *6.4.4.3 Vitamin D therapy and disease progression by relapse*

Six studies investigated the effect of vitamin D therapy on the progression of MS measured by relapses. Three of these studies used $25(OH)D_3$ as the intervention, and found that patients in the treatment groups had significantly fewer relapses (Burton et al., 2010; Goldberg et al., 1986; Pierrot-Deseilligny et al., 2012). These trials were conducted in North America and France. Another study, with the same finding, was conducted in Israel and used alfacalcidol (Achiron et al., 2015). The other two trials found no differences in relapse rate between the treatment and control groups (Kampman et al., 2012; Soilu-Hänninen et al., 2012). Both of these trials also used $25(OH)D_3$, and came from Norway and Finland respectively.

Of the trials that found a negative association between supplementation and relapse, one was a randomised, double-blind, placebo controlled trial of 158 participants with an intervention duration of 26 weeks (Achiron et al., 2015). The designs of the other studies were less methodologically rigorous. Goldberg et al.'s (1986) experimental study comprised 16 MS patients who also served as their own controls with data drawn from their case histories. One further observational, uncontrolled study of 156 RRMS patients over 26 weeks, included in this section because the exposure was externally manipulated, found that the incidence rate ratio of

relapses decreased as 25(OH)$D_3$ increased. However, this effect plateaued above 110nmol/L (Pierrot-Deseilligny et al., 2012). A final randomised, open-label trial of 49 participants supplemented both groups with either 40,000 or 4000 IU per day, and ran for 52 weeks.

The two trials that found no effect of vitamin D supplementation on relapse rate comprised sample sizes of between 32 and 35 in each group, and both administered 20,000 IU of vitamin $D_3$ per week. Both studies were randomised, double-blind, placebo controlled trials, and ran over a period of 96 weeks (Kampman et al., 2012) and 52 weeks (Soilu-Hänninen et al., 2012). Perhaps crucially, those trials that observed a protective effect of vitamin D tended to use larger or more regular doses of vitamin D.

#### *6.4.4.4 Vitamin D therapy and disease progression by MRI*

Four studies explored whether vitamin D therapy has an effect on disease progression by MRI activity. Two studies involved treatment versus placebo groups, and both were year-long trials of 25(OH)$D_3$ administered weekly (Soilu-Hänninen et al., 2012; Derakhshandi et al., 2013). Both studies, from Finland and Iran, found that there were significantly fewer lesions detected by MRI in the treatment group.

Two further studies, both conducted over 26 weeks, found no differences in new MRI-detected lesions between groups. One of these studies involved a high-dose versus low-dose group, with 25(OH)$D_2$ administered daily (Stein et al., 2011). This same study found that a high dose was associated with better EDSS scores. The other study involved 25(OH)$D_3$ supplied to the treatment group each month, and a placebo group (Mosayebi et al., 2011). The duration of the intervention possibly accounts for the difference in findings; additionally, frequency of supplementation and a difference between vitamins $D_2$ and $D_3$ may be important.

Table 6.14 Studies involving vitamin D interventions

| | Finding | Study design | Sample size | Intervention | Intervention duration | Authors |
|---|---|---|---|---|---|---|
| **Safety and tolerability of vitamin D therapy in MS** | Vitamin D is well-tolerated in diet-compliant patients | Open-label pilot trial | 15 RRMS patients | $1,25(OH)_2D$ (2.5g/day) | 48 weeks | (Wingerchuk et al., 2005) |
| | $25(OH)D_3$ is safe and tolerable in patients with MS | Double-blind, single centre, RCT | 20 high dose, 20 low dose | $25(OH)D_3$ (High dose 10,400 IU/day; low dose 800 IU/day) | 26 weeks | (Sotirchos et al., 2016) |
| **Vitamin D levels in MS patients following vitamin D therapy** | Vitamin D levels rose significantly in the high treatment group compared to the low treatment group | Double-blind RCT | 11 high-dose; 12 low dose (3 withdrawals) | $25(OH)D_2$ (High-dose 1,000 IU/day + supplement; low dose 1,000 IU/day + placebo) | 26 weeks | (Stein et al., 2011) |
| | | Retrospective evaluation of serum vitamin D | 49 MS patients examined for change in their 25(OH)D levels | High dose $25(OH)D_2$ (50,000 IU/day) Low dose $25(OH)D_3$ (≤800 IU/day) | Low or high dose for 7-10 days followed by weekly or bi-weekly supplementation for at least 3 months | (Hiremath et al., 2009) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Vitamin D levels rose significantly in the treatment group compared to placebo | Double-blind, placebo-controlled RCT | 36 treatment, 32 controls | 25(OH)$D_3$ (20,000 IU/week) | 96 weeks | (Røsjø et al., 2015) |
| | Vitamin D levels were sufficient in the treatment group year-round but only increased in the summer in the placebo group | Double-blind placebo controlled RCT | 35 treatment; 33 placebo | 25(OH)$D_3$ (20,000 IU/week) | 96 weeks | (Steffensen et al., 2013) |
| **Prevention or delay to CDMS by vitamin D therapy** | Vitamin D treatment may prevent conversion from ON to CDMS | Double-blind, randomised, parallel-group trial | 13 treatment; 11 placebo | 25(OH)$D_3$ (50,000 IU/week) | 52 weeks | (Derakhshandi et al., 2013) |
| **Effect of vitamin D on disease progression by EDSS score** | High-dose treatment group had improved EDSS compared to low-dose treatment group | Double-blind RCT | 11 high-dose; 12 low dose (3 withdrawals) | 25(OH)$D_2$ (High-dose 1,000 IU/day + supplement; low dose 1,000 IU/day + placebo) | 26 weeks | (Stein et al., 2011) |
| | There were no significant differences on EDSS between treatment and placebo groups | Double-blind placebo-controlled RCT | 28 treatment; 34 placebo | 25(OH)$D_3$ (300,000 IU/month) | 26 weeks | (Mosayebi et al., 2011) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | Double-blind, RCT | 35 treatment; 33 placebo | 25(OH)$D_3$ (20,000 IU/week) | 96 weeks | (Kampman et al., 2012) |
| **Effect of vitamin D on disease progression by relapse** | Treatment group patients had fewer relapses than controls | Randomised, open-label trial | 25 treatment; 24 control | 25(OH)$D_3$ (treatment up to 40,000 IU/day; controls ≤4,000 IU/day) | 52 weeks | (Burton et al., 2010) |
| | | Observational, uncontrolled study | 156 RRMS patients | 25(OH)$D_3$ (up to 100,000 IU/month depending on baseline) | 26 weeks | (Pierrot-Deseilligny et al., 2012) |
| | | Double-blind placebo controlled RCT | 80 treatment; 78 placebo | Alfacalcidol (1 mcg/day) | 26 weeks | (Achiron et al., 2015) |
| | | Experimental | 16 MS patients | 25(OH)$D_3$ (5,000 IU/day) | 96 weeks | (Goldberg et al., 1986) |
| | There were no significant differences on relapse between the treatment and placebo groups | Double-blind, controlled RCT | 35 treatment; 33 placebo | 25(OH)$D_3$ (20,000 IU/week) | 96 weeks | (Kampman et al., 2012) |
| | | Double-blind, placebo controlled, RCT | 34 treatment, 32 placebo | 25(OH)$D_3$ (20,000 IU/week) | 52 weeks | (Soilu-Hänninen et al., 2012) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Effect of vitamin D on disease progression by MRI activity** | Treatment group had fewer T1 lesions on MRI than placebo group | Double-blind, placebo controlled, RCT | 34 treatment, 32 placebo | 25(OH)$D_3$ (20,000 IU/week) | 52 weeks | (Soilu-Hänninen et al., 2012) |
| | Treatment group had significantly fewer MRI lesions than the placebo group | Double-blind, randomised, parallel-group trial | 13 treatment; 11 placebo | 25(OH)$D_3$ (50,000 IU/week) | 52 weeks | (Derakhshandi et al., 2013) |
| | High-dose treatment group did not have reduced MRI activity compared to low-dose treatment group | Double-blind RCT | 11 high-dose; 12 low dose (3 withdrawals) | 25(OH)$D_2$ (High-dose 1,000 IU/day + supplement; low dose 1,000 IU/day + placebo) | 26 weeks | (Stein et al., 2011) |
| | There were no significant differences in gadolinium-enhancing lesions between treatment and placebo groups | Double-blind RCT | 28 treatment; 34 placebo | 25(OH)$D_3$ (300,000 IU/month) | 26 weeks | (Mosayebi et al., 2011) |

### 6.4.5 Summary

We identified fourteen intervention studies that directly explored the effect of vitamin D supplementation on people with MS. From these studies, four themes emerged:

1. **Safety and tolerability of vitamin D therapy**: both studies found that vitamin D is indeed safe and well-tolerated, however these studies do not seek to understand whether vitamin D may be causally involved in the pathogenesis of MS or therapeutic as a treatment
2. **Vitamin D levels following vitamin D therapy:** studies were all in agreement that supplementation, and with higher doses, were effective at raising blood vitamin D in MS. Again, these studies did not question a biologically causative or therapeutic role of vitamin D in MS
3. **Conversion or delay from ON to CDMS:** one study sought to identify whether treatment with vitamin D may delay or prevent the conversion of ON to CDMS, finding a positive effect. Replication of this study with a greater sample size and in other study populations would lend support to a possible causal role of vitamin D in MS onset
4. **Effect of vitamin D on disease progression:** progression was variously categorised by EDSS, relapse and MRI activity, and findings were inconsistent. Reasons for the heterogeneity of results include the multiple types and amounts of vitamin D administered, varying sample sizes, lengths of intervention, and different study designs

Whilst interventional studies are useful for reducing the environmental noise present in observational studies, one further method of detecting associations between exposure and disease is through genetic studies.

## 6.5 Results: Genetic Studies

The genetic studies comprised genetic association and genome-wide association (GWA) studies. The most frequently used design was genetic association, and these were conducted using case control and family designs (Table 6.15). We found only three GWA studies. The 'other' column in Table 6.15 included twin studies, and whole exome sequencing of MS cases from multicase families.

Most studies defined MS according to the McDonald or Poser criteria. Others state that MS has been neurologist- or MRI-confirmed, although specific criteria are not named. Only one study failed to state how MS had been identified and confirmed (Table 6.16).

Most studies were conducted in Europe, The Americas and the Western Pacific regions (Table 6.17). Once again, the only region which is not represented is Africa.

Tables 6.18 and onwards are presented according to findings by genotype. Where possible, SNPs that have been explored in multiple studies are presented together for ease of comparison. Sample sizes are reported in the tables.

Table 6.15 Genetic studies summary of study designs

| | Study design | | | | | | |
|---|---|---|---|---|---|---|---|
| **Aim** | **GWAS** | **Case-control** | **Family association study** | **Prospective cohort study** | **Cross-sectional** | **Nested case-control study** | **Other** |
| To identify MS susceptibility loci | 1 | - | - | - | - | - | - |
| To evaluate the role of genes in 25(OH)D and MS | - | 21 | 3 | - | 2 | - | 1 |
| To evaluate the role of genes involved in 25(OH)D and MS risk, onset and clinical course | 2 | 13 | 1 | 3 | - | 3 | 2 |
| To evaluate the association between genes and serum 25(OH)D levels in MS | - | 6 | - | 3 | - | 1 | 3 |
| To evaluate the association between vitamin D genes and UV exposure in patients with MS | - | 6 | - | - | 1 | - | - |

Table 6.16 Summary of MS criteria used

| | Criteria | | | | | |
|---|---|---|---|---|---|---|
| **Aim** | **Schumacher** | **Poser** | **McDonald** | **Neurologist confirmed/MRI** | **No info** | **Other** |
| To identify MS susceptibility loci | - | 1 | 1 | - | - | - |
| To evaluate the role of genes in 25(OH)D and MS | - | 4 | 19 | 5 | 1 | - |
| To evaluate the role of genes involved in 25(OH)D and MS risk, onset and clinical course | - | 8 | 17 | 6 | - | - |
| To evaluate the association between genes and serum 25(OH)D levels in MS | - | 2 | 6 | 4 | - | - |
| To evaluate the association between vitamin D genes and UV exposure in patients with MS | - | 7 | - | 3 | - | - |

Table 6.17 Distribution of studies geographically

| | Study region (according to WHO) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Aim** | **Africa** | **The Americas** | **South-East Asia** | **Europe** | **Eastern Mediterranean** | **Western Pacific** | **No info/ other** |
| To identify MS susceptibility loci | - | - | - | - | - | 1 | - |
| To evaluate the role of genes in 25(OH)D and MS | - | 3 | 2 | 12 | 6 | 8 | - |
| To evaluate the role of genes involved in 25(OH)D and MS risk, onset and clinical course | - | 7 | - | 12 | 2 | 6 | 3 |
| To evaluate the association between genes and serum 25(OH)D levels in MS | - | 4 | - | 3 | 2 | 3 | - |
| To evaluate the association between vitamin D genes and UV exposure in patients with MS | - | - | - | 4 | - | 3 | - |

### 6.5.1 Identifying susceptibility loci in MS (Table 6.18)

One study aimed to find new susceptibility loci in MS (Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene), 2009). Using 1618 MS cases of European ancestry from Australia and New Zealand, and 3413 controls from the UK and people with European ancestry from the USA, two new susceptibility loci in chromosomes 12 and 20 were identified and subsequently replicated in an independent dataset. On chromosome 20q13 two of the identified SNPs were in complete linkage disequilibrium with two other SNPs (i.e. the alleles have not been separated by recombination) which are associated with susceptibility to other autoimmune diseases. However, on chromosome 12q13-14, the authors hypothesise the *CYP27B1* may be the causal gene. This gene encodes the enzyme which converts 25(OH)D into the bioactive $1,25(OH)_2D$, which has important immune functions (Prietl et al., 2013) and has since been linked to MS (Ramagopalan et al., 2011).

Table 6.18 Studies aiming to identify susceptibility loci in MS

| | Finding | Study design | Diagnostic criteria | Study region | Measures | Authors |
|---|---|---|---|---|---|---|
| **To identify susceptibility loci in MS** | New susceptibility loci for MS on chromosomes 12 and 20 were identified. | GWAS | Poser/McDonald | Europe and people of European ancestry in Western Pacific and Americas regions | rs703842 (*CYP27B1*)<br>rs10876994 (*CYP27B1*)<br>rs12368653 (*AGAP2*)<br>rs6074022 (*CD40*)<br>rs1569723 (*CD40*)<br>rs6131010 (*CD40*) | (Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene), 2009) |

### 6.5.2 The role of genes in vitamin D and MS (Table 6.19)

#### *The role of VDR variants in MS*

Eighteen studies investigated general associations between MS and *VDR* variants. *VDR* SNPs comprised rs731236 (*TaqI*), rs7975232 (*ApaI*), rs1544410 (*BsmI*) and rs10735810, now merged into rs2228570 (*FokI*). The VDR is required for cellular response to $1,25(OH)_2D_3$, and all tissues that directly respond to $1,25(OH)_2D_3$ contain the VDR. Mutations in the *VDR* gene may prevent the expression of VDR, or may reduce its efficacy, leading to partial or total resistance to $1,25(OH)_2D_3$ (Pike et al., 2011).

Two of the six studies that explored rs731236 (*TaqI*) found no evidence of linkage between rs731236 and MS (Steckley et al., 2000), or no differences between cases and controls in allele or genotype frequency (Garcia-Martin et al., 2013). However, the other four studies found evidence of allelic and genotypic differences between cases and controls.

Two of these studies, from the Western Pacific and Europe, and Eastern Mediterranean, found that cases had a higher frequency of C alleles (Cox et al., 2012b; Narooie-Nejad et al., 2015a). The former had a considerably larger sample size. However, a third study found that cases had a lower frequency of C alleles compared to controls (Ben-Selma et al., 2015). This study, from South East Asia, again had a relatively small sample size.

The fourth study that found a significant difference between genotype and allele frequency was from the Western Pacific, and found a higher frequency of t alleles in cases (Tajouri et al., 2005). Ben-Selma et al. (2015) did not find any differences in genotype frequencies, however they did find that T alleles were positively associated with MS. Conversely, Narooie-Nejad et al. (2015a) found that T alleles were negatively associated with MS. Findings are overall inconsistent, although some effects have been

replicated in different populations. Small sample sizes which may be underpowered may contribute to the lack of agreement.

Five papers looked at rs7975232 (*ApaI*). Findings ranged from no evidence of linkage between *VDR* variant rs7975232 and MS (Steckley et al., 2000) and no differences in genotype or allelic frequencies in cases and controls (Ben-Selma et al., 2015), to significant associations and differences in genotype and allele frequencies (Tajouri et al., 2005; Niino et al., 2000; Narooie-Nejad et al., 2015a). Once again, there was no consistency in findings among studies that reported differences between cases and controls; moreover each of these three studies defined MS according to different criteria.

Rs2228570 (*FokI*) was explored in five studies. Four of these studies came from three world regions, and found no associations between rs2228570 and MS (Smolders et al., 2009b; Narooie-Nejad et al., 2015b; Cox et al., 2012b; Garcia-Martin et al., 2013). One of these studies had a comparatively large sample size (Cox et al., 2012b). There was however a statistically insignificant suggestion of increasing MS risk in people who were homozygous for the *HLA-DRB1*1501* allele and rs2228570 (Cox et al., 2012a). Only one study found a genotype difference in cases compared to controls (Cierny et al., 2015).

The two studies that looked at rs1544410 (*BsmI*) had contrasting results. One study found that cases had a significantly lower frequency of bb genotypes and b alleles compared to controls (Narooie-Nejad et al., 2015b); the other found that cases had significantly higher frequency of bb genotypes and b alleles compared to controls (Fukazawa et al., 1999). These studies also defined MS according to different criteria, comprised small sample sizes which may contribute to the contradictory findings, and originated from different world regions, making direct comparisons difficult.

### *The role of DBP genes in MS*

Two papers examined the role of DBP genes (Steckley et al., 2000; Niino et al., 2002). Among its other functions, DBP is involved in binding circulating vitamin D for transport to the liver, kidney, bone, and other target tissues (Uitterlinden, 2011). DBP is encoded by the gene *Gc*, and the two most common DBP SNPs are rs7041 and rs4588. Combinations of these two SNPs result in six DBP isotypes where differences in protein configuration lead to differences in vitamin D binding affinity. Aspartic acid and threonine, and glutamic acid and threonine, both correspond to *Gc1* which has the strongest binding affinity; aspartic acid and lysine corresponds to *Gc2* and has the lowest binding affinity (Braithwaite et al., 2015; Uitterlinden, 2011). Two restriction enzymes, where amino acid substitutions had been identified, were explored: *HaeIII,* located at codon 416 (rs7041) and *StyI*, located at codon 420 (rs4588). Neither paper found any evidence of an association between DBP and MS, however the sample sizes were again very small for association studies (Steckley et al., 2000; Niino et al., 2002).

### *The role of vitamin D metabolism genes in MS*

Six papers explored vitamin D metabolism genes and MS, all of which looked at *CYP* genes. *CYP27B1* encodes the enzyme 25-hydroxyvitamin $D_3$ 1-alpha hydroxylase which catalyses the conversion of calcidiol to calcitriol; *CYP27A1* is also involved in vitamin D metabolism although its role is more controversial (Jones and Prosser, 2011). One paper looked at one *CYP27A1* and several *CYP27B1* SNPs. They found no association between the *CYP27A1* SNP and MS in their Chinese study population (Zhuang et al., 2015). The majority of papers however studied *CYP27B1* with diverse results. Three studies found significant associations between MS and some or all of the SNPs under study (Sundqvist et al., 2010; Ramagopalan et al., 2011; Zhuang et al., 2015), while others failed to find any significant associations (Ban et al., 2013; Barizzone et al., 2013; Steckley et al., 2000). Four of these studies looked at *CYP27B1*

SNP rs118204009, one of which used whole genome sequencing. They found that the SNP was present in all affected family members; furthermore, it showed an association with MS in a large MS cohort in their North American population (Ramagopalan et al., 2011). However, the studies that attempted to replicate this finding, all of which were case-control designs from Europe and the Western Pacific, failed to find evidence of an association (Zhuang et al., 2015; Ban et al., 2013; Barizzone et al., 2013). There is however again a lack of consistency over the diagnostic criteria.

Table 6.19 Studies evaluating the role of genes in vitamin D and MS

| | Finding | Study design | Diagnostic criteria | Study region | Measures | Sample size | Authors |
|---|---|---|---|---|---|---|---|
| **To evaluate the role of *VDR* variants in MS** | There was no evidence for linkage of candidate gene *VDR* with MS in the Canadian population | Family-based association | Neurologist-confirmed | The Americas | rs731236 (*TaqI*) | 236 sibling pairs from 187 families | (Steckley et al., 2000) |
| | There was no difference in genotype or allele frequencies between cases and controls | Case-control association | McDonald | Europe | rs731236 (*TaqI*) | 303 cases 310 controls | (Garcia-Martin et al., 2013) |
| | Cases had a higher frequency of C alleles compared to controls | Family-based and case-control association | McDonald | Western Pacific/Europe | rs731236 (*TaqI*) | 1153 trio families 726 cases 604 controls | (Cox et al., 2012a) |
| | Cases had higher frequency of C alleles compared to controls; T allele showed negative association with MS | Case-control association | McDonald | Eastern Mediterranean | rs731236 (*TaqI*) | 113 cases 122 controls | (Narooie-Nejad et al., 2015a) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Cases had higher frequency of T alleles, particularly ages 15-24, and lower frequency of C alleles compared to controls but not different genotype frequencies | Case-control association | McDonald | South-East Asia | rs731236 (*TaqI*) | 60 cases<br>114 controls | (Ben-Selma et al., 2015) |
| | Cases had higher frequency of t alleles compared to controls, and genotype frequencies were significantly different | Case-control association | Neurologist-confirmed | Western Pacific | rs731236 (*TaqI*) | 104 cases<br>104 controls | (Tajouri et al., 2005) |
| | There was no evidence for linkage of candidate gene *VDR* with MS in the Canadian population | Family-based association | Neurologist-confirmed | The Americas | rs7975232 (*ApaI*) | 236 sibling pairs from 187 families | (Steckley et al., 2000) |
| | There was no difference in genotype or allele frequencies between cases and controls | Case-control association | McDonald | South-East Asia | rs7975232 (*ApaI*) | 60 cases<br>114 controls | (Ben-Selma et al., 2015) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Allele but not genotype frequencies were associated with MS | Case-control association | Neurologist-confirmed | Western Pacific | rs7975232 (*ApaI*) | 104 cases<br>104 controls | (Tajouri et al., 2005) |
| | Cases had higher frequency of AA genotypes and [A] alleles compared to controls | Case-control association | Poser | Western Pacific | rs7975232 (*ApaI*) | 77 cases<br>95 controls | (Niino et al., 2000) |
| | Cases had higher frequency of CC genotypes compared to controls | Case-control association | McDonald | Eastern Mediterranean | rs7975232 (*ApaI*) | 113 cases<br>122 controls | (Narooie-Nejad et al., 2015a) |
| | There was no difference in genotype or allele frequencies between cases and controls | Case-control association | McDonald | Europe | rs2228570 (*FokI*) | 303 cases<br>310 controls | (Garcia-Martin et al., 2013) |
| | There was no association between *FokI* and MS | Case-control association | McDonald | Eastern Mediterranean | rs2228570 (*FokI*) | 113 cases<br>122 controls | (Narooie-Nejad et al., 2015b) |
| | | | | Western Pacific/ Europe | rs2228570 (*FokI*) | 1153 trio families<br>726 cases<br>604 controls | (Cox et al., 2012a) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | Europe | 25(OH)D<br>1,25(OH)$_2$D<br>rs2228570<br>(*FokI*) | 212 cases<br>289 controls | (Smolders et al., 2009b) |
| | Cases had a higher frequency of Ff genotype compared to controls | Case-control association | McDonald | Europe | rs2228570<br>(*FokI*) | 270 cases<br>303 controls | (Cierny et al., 2015) |
| | Cases had a significantly lower frequency of bb genotypes and b alleles compared to controls | Case-control association | McDonald | Eastern Mediterranean | rs1544410<br>(*BsmI*) | 113 cases<br>122 controls | (Narooie-Nejad et al., 2015b) |
| | Cases had significantly higher frequency of bb genotypes and b alleles compared with controls | Case-control association | Poser | Western Pacific | rs1544410<br>(*BsmI*) | 77 cases<br>95 controls | (Fukazawa et al., 1999) |
| **To evaluate the role of *DBP* genes and MS** | There was no evidence for linkage of candidate genes with MS in the Canadian population | Family-based association | Neurologist-confirmed | The Americas | HaeIII<br>StyI | 236 sibling pairs from 187 families | (Steckley et al., 2000) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | There was no association between DBP polymorphisms and MS occurrence | Case-control association | McDonald | Western Pacific | In DBP gene: Codon 416 (*HaeIII*) Codon 420 (*StyI*) | 77 cases 95 controls | (Niino et al., 2002) |
| **To evaluate the role of vitamin D metabolism genes and MS** | Significant associations were found between SNPs and MS | Nested case-control | McDonald | Europe | rs4646536 (*CYP27B1*) rs10877012 (*CYP27B1*) rs10877015 (*CYP27B1*) rs703842 (*CYP27B1*) | 2158 cases 1759 controls | (Sundqvist et al., 2010) |
| | Rare loss of function variants in *CYP27B1* confer significant risk of MS | Whole exome sequencing | Poser | The Americas | *CYP27B1*: rs118204009 rs118204012 rs118204011 | 1 affected individual from each of 43 multicase families | (Ramagopalan et al., 2011) |
| | Significant associations were found between variant* and MS, and between variants* & ** and NMO | Case-control association | McDonald | Western Pacific | rs12368653 (*CYP27B1*) rs703842*;** (*CYP27B1*) rs10876994** (*CYP27B1*) rs118204009 (*CYP27B1*) rs2248359 (*CYP27A1*) | 161 MS cases 110 NMO cases 301 controls | (Zhuang et al., 2015) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | No evidence to suggest that mutant *CYP27B1* alleles influence risk of developing MS | Family-based and case-control association | No info | Europe | rs118204009 (*CYP27B1*) | 495 multicase families<br>2092 single case families<br>4594 cases<br>3583 controls | (Ban et al., 2013) |
| | | Case-control association | Poser/ McDonald | Europe | rs118204009 (*CYP27B1*) | 1 MS cases from each of 134 multicase families<br>1692 MS cases<br>989 controls | (Barizzone et al., 2013) |
| | There was no evidence for an association between *CYP27B1* and MS in the Canadian population | Family-based association | Neurologist -confirmed | The Americas | *CYP27B1* | 236 sibling pairs from 187 families | (Steckley et al., 2000) |

### 6.5.3 The role of genes in vitamin D and MS risk, onset, and clinical course (Table 6.20)

#### *The role of VDR variants in 25(OH)D and MS risk, onset, and clinical course*

Sixteen papers explored *VDR* variants and MS, eight of which were concerned with rs731236 (*TaqI*). Two of these six studies found that different rs731236 alleles were associated with an increased risk of MS (Al-Temaimi et al., 2015) or progressive MS (Tajouri et al., 2005).Another study found that rs731236 is associated with a reduced risk of MS when it co-segregates with *HLA-DRB1*1501* (Agliardi et al., 2011). This same paper also found that rs731236 modulates *VDR* expression in *HLA-DRB1*1501*-positive individuals (Agliardi et al., 2011). A further paper found that although there was no association between rs731236 and MS risk, the presence of rs731236 slightly modulates the risk of MS conferred by the presence of HLA-DRB1*1501 (Irizar et al., 2012). Four additional studies found no association between rs731236 and MS risk (Partridge et al., 2004; Simon et al., 2010b; Garcia-Martin et al., 2013; Sioka et al., 2011). Furthermore, no associations were found between rs731236 and disease progression (Al-Temaimi et al., 2015), or onset age or clinical course of MS (Garcia-Martin et al., 2013). Sample sizes, study designs, and diagnostic criteria varied across these studies.

Three papers looked at rs7975232 (*ApaI*). Three findings showed that there was no association between rs7975232 and MS risk (Al-Temaimi et al., 2015; Simon et al., 2010b; Irizar et al., 2012). One study found that the presence of rs7975232 modulates the risk of MS conferred by the presence of HLA-DRB1*1501 (Irizar et al., 2012). Additionally, the AA genotype was associated with low disease progression, characterised by a low EDSS score (Al-Temaimi et al., 2015).

Six papers looked at rs2228570 (*FokI*). One study explored the interaction between rs2228570 and *HLA1-DRB1*1501,*reporting a statistically insignificant trend

of increasing risk between rs2228570 and individuals who were homozygous for *HLA-DRB1*1501* (Cox et al., 2012b). A further study found a protective effect against MS in ff genotypes (Partridge et al., 2004), and another found that the ff genotype frequency was lower in people with more advance disease (Mamutse et al., 2008). None of the remaining studies found any associations between rs2228570 and MS risk (Garcia-Martin et al., 2013; Simon et al., 2010b; Al-Temaimi et al., 2015), rs2228570 and disease progression (Al-Temaimi et al., 2015), or rs2228570 and onset age or clinical course (Garcia-Martin et al., 2013). These studies all explored slightly different hypotheses and used different terms to define MS, and therefore replication is not really achieved. However, there is some consistency in a lack of effect between rs2228570 and MS across several different populations.

Three papers explored rs1544410 (*BsmI*) and MS. Although one study found that there was an increased risk of MS in carriers of the C allele (Al-Temaimi et al., 2015), three other findings showed no association with risk (Simon et al., 2010b; Sioka et al., 2011) and no association with disease progression (Al-Temaimi et al., 2015). Two further *VDR* SNPS, rs2254210 and rs98784, were explored in one twin study (Orton et al., 2008). There was no association with MS.

### *The role of DBP genes in 25(OH)D and MS risk, onset, and clinical course*

Two papers were concerned with the role of DBP genes in vitamin D and MS. Additionally, a GWA study found that although vitamin D may have beneficial effects on the risk of MS, these effects may be reduced in the presence of the *HLA-DRB1*1501* allele (Simon et al., 2011). Two further studies found no association between two DBP SNPs and MS risk in the US (Simon et al., 2010b), or DBP and age of onset in a Japanese population (Niino et al., 2002).

### *The role of vitamin D metabolising genes in 25(OH)D and MS risk, onset, and clinical course*

Three papers were concerned with vitamin D metabolism genes and MS. Simon et al. (2011) found that the presence of *HLA-DRB1*1501* alongside certain vitamin D metabolising genes, reduces any potential beneficial effect of vitamin D on MS risk. SNP rs2248359 in gene *CYP24A1*, which has a role in the decline of circulating vitamin D, was found to modify the relationship between 25(OH)D and the hazard of relapse in a prospective cohort study, however the finding was not statistically significant after adjustment for multiple testing (Lin et al., 2014a). The final paper found no association between vitamin D metabolising SNPs and MS risk (Simon et al., 2010b). This literature presents an ambiguous picture with multiple genes and SNPs tested across multiple populations, with different diagnostic criteria.

### *The role of other genes in 25(OH)D and MS risk, onset, and clinical course*

Other genes which did not fit into the above categories include 7-dehydrocholesterol reductase which is on the vitamin D pathway, the protein kinase family of genes which are involved in vitamin D gene signalling, and the human leukocyte antigen complex, the *DRB1*1501* allele of which increases MS risk.

Further SNPs studied by Lin et al. (2014a) modified the relationship between 25(OH)D and hazard of relapse. This association was however not significant after adjustment for multiple testing.

The final two studies in this category looked at various *HLA-DRB1* alleles. One study found that alleles that expressed non-responsive *VDRE* motifs were significantly associated with reduced MS risk, suggesting that vitamin D regulation of *HLA-DR* expression is important (Nolan et al., 2012). The other study, however, found that *VDRE* motif variation did not independently contribute to MS risk (Cocco et al., 2012).

Table 6.20 Studies evaluating the role of genes in vitamin D and MS risk, onset and clinical course

| Genetic studies | Finding | Study design | Diagnostic criteria | Study region | Measures | Sample size | Authors |
|---|---|---|---|---|---|---|---|
| **To evaluate the role of *VDR* variants involved in 25(OH)D and MS risk, onset and clinical course** | *TaqI* G allele is associated with increased MS risk | Case-control association | Neurologist-confirmed | Eastern Mediterranean | 25(OH)D rs731236 (*TaqI*) | 50 cases 50 controls | (Al-Temaimi et al., 2015) |
| | *TaqI* t allele is associated with increased risk of progressive MS | Case-control association | Neurologist-confirmed | Western Pacific | rs731236 (*TaqI*) | 104 cases 104 controls | (Tajouri et al., 2005) |
| | *TaqI* T and C haplotypes are associated with reduced risk of MS when SNPs co-segregate with the *HLA-DRB1*1501* allele | Case-control association | McDonald | Europe | rs731236 (*TaqI*) | 641 cases 558 controls | (Agliardi et al., 2011) |
| | *TaqI* TT genotype modulates *VDR* expression and is protective against MS in *HLA-DRB1*1501* +ve individuals | Case-control association | McDonald | Europe | *HLA-DRB1*1501* rs731236 (*TaqI*) | 641 cases 558 controls | (Agliardi et al., 2011) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | There was no association between *TaqI* and MS risk | Case-control association | Poser | Europe | rs731236 (*TaqI*) | 419 cases 422 controls | (Partridge et al., 2004) |
| | | Nested case-control | Poser | The Americas | rs731236 (*TaqI*) | 214 cases 428 controls | (Simon et al., 2010b) |
| | | Case-control association | McDonald | Europe | rs731236 (*TaqI*) | 364 cases 513 controls | (Irizar et al., 2012) |
| | | | | | | 61 cases 81 controls | (Sioka et al., 2011) |
| | There was no association between *TaqI* SNP and disease progression | Case-control association | Neurologist-confirmed | Eastern Mediterranean | 25(OH)D rs731236 (*TaqI*) | 50 cases 50 controls | (Al-Temaimi et al., 2015) |
| | *TaqI* SNP was not related to age at MS onset or clinical course | Case-control association | McDonald | Europe | rs731236 (*TaqI*) | 303 cases 310 controls | (Garcia-Martin et al., 2013) |
| | *TaqI* SNP slightly modulates MS risk conferred by HLA-DRB1*1501 | Case-control association | McDonald | Europe | rs731236 (*TaqI*) | 364 cases 513 controls | (Irizar et al., 2012) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *ApaI* AA genotype is associated with low disease progression | Case-control association | Neurologist-confirmed | Eastern Mediterranean | 25(OH)D rs7975232 (*ApaI*) | 50 cases 50 controls | (Al-Temaimi et al., 2015) |
| | There was no association between *ApaI* SNPs and MS risk | Case-control association | Neurologist-confirmed | Eastern Mediterranean | 25(OH)D rs7975232 (*ApaI*) | 50 cases 50 controls | (Al-Temaimi et al., 2015) |
| | | Nested case-control | Poser | The Americas | rs7975232 (*ApaI*) | 214 cases 428 controls | (Simon et al., 2010b) |
| | | Case-control association | McDonald | Europe | rs731236 (*TaqI*) | 364 cases 513 controls | (Irizar et al., 2012) |
| | *ApaI* SNP slightly modulates MS risk conferred by HLA-DRB1*1501 | Case-control association | McDonald | Europe | rs731236 (*TaqI*) | 364 cases 513 controls | (Irizar et al., 2012) |
| | There is a non-statistically significant trend of increasing MS risk in those who were homozygous for *HLA-DRB1*1501* | Case-control association | McDonald | Western Pacific/Europe | rs2228570 (*FokI*) | 1153 trio families 726 cases 604 controls | (Cox et al., 2012a) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *FokI* ff genotype was associated with reduced MS risk | Case-control association | Poser | Europe | rs2228570 (*FokI*) | 419 cases 422 controls | (Partridge et al., 2004) |
| | | Nested case-control | Poser | The Americas | rs2228570 (*FokI*) | 214 cases 428 controls | (Simon et al., 2010b) |
| | *FokI* was not associated with MS risk | Case-control association | Neurologist-confirmed | Eastern Mediterranean | 25(OH)D rs2228570 (*FokI*) | 50 cases 50 controls | (Al-Temaimi et al., 2015) |
| | *FokI* was not associated with disease progression | Case-control association | Neurologist-confirmed | Eastern Mediterranean | 25(OH)D rs2228570 (*FokI*) | 50 cases 50 controls | (Al-Temaimi et al., 2015) |
| | *FokI* ff genotype frequency was lower in cases with disease progression of EDSS >=6 | Cross-sectional | Poser | Europe | $Ff^{30875}$ | 512 cases | (Mamutse et al., 2008) |
| | *FokI* was not related to age at MS onset or clinical course | Case-control association | McDonald | Europe | rs2228570 (*FokI*) | 303 cases 310 controls | (Garcia-Martin et al., 2013) |
| | *BsmI* C allele was associated with increased MS risk | Case-control association | Neurologist-confirmed | Eastern Mediterranean | 25(OH)D rs1544410 (*BsmI*) | 50 cases 50 controls | (Al-Temaimi et al., 2015) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | There was no association between *BsmI* SNP and MS risk | Nested case-control | Poser | The Americas | rs1544410 (*BsmI*) | 214 cases 428 controls | (Simon et al., 2010b) |
| | | Case-control association | McDonald | Europe | rs1544410 (*BsmI*) | 61 cases 81 controls | (Sioka et al., 2011) |
| | *BsmI* SNP was not associated with disease progression | Case-control association | Neurologist-confirmed | Eastern Mediterranean | 25(OH)D rs1544410 (*BsmI*) | 50 cases 50 controls | (Al-Temaimi et al., 2015) |
| | *VDR* SNPs were not associated with MS status | Twin | Neurologist-confirmed | The Americas | 25(OH)D rs2254210 rs98784 | 198 twin persons (16 pairs concordant for MS) | (Orton et al., 2008) |
| **To evaluate the role of *DBP* genes involved in 25(OH)D and MS risk, onset and clinical course** | Possible beneficial effects of vitamin D on MS risk may be attenuated by carrying the *HLA-DR15* MS risk allele | GWAS | McDonald | The Americas; Europe | 25(OH)D rs7041 | 1655 cases 6349 controls | (Simon et al., 2011) |
| | There was no association between DBP SNPs and MS risk | Nested case-control | Poser | The Americas | rs4588 rs7041 | 214 cases 428 controls | (Simon et al., 2010b) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | There was no association between DBP polymorphisms and age of MS onset | Case-control association | McDonald | Western Pacific | In DBP gene: Codon 416 (rs7041) Codon 420 (rs4588) | 77 cases 95 controls | (Niino et al., 2002) |
| **To evaluate the role of vitamin D metabolising genes involved in 25(OH)D and MS risk, onset and clinical course** | Possible beneficial effects of vitamin D on MS risk may be attenuated by carrying the *HLA-DR15* MS risk allele | GWAS | McDonald | The Americas; Europe | 25(OH)D rs10741657 (*CYP2R1*) | 1655 cases 6349 controls | (Simon et al., 2011) |
| | SNPs modified the relationship between 25(OH)D and hazard of relapse but did not reach significance | Prospective cohort study | McDonald | Western Pacific | 25(OH)D rs2248359 (*CYP24A1*) | 141 RRMS cases | (Lin et al., 2014a) |
| | There was no association between vitamin D metabolising SNPs and MS risk | Nested case-control | Poser | The Americas | rs703426 rs10877012 (*CYP27B1*) rs2296241 (*CYP24A1*) rs10500804 rs12794714 (*CYP2R1*) | 214 cases 428 controls | (Simon et al., 2010b) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **To evaluate the role of other genes involved in 25(OH)D and MS risk, onset and clinical course** | SNPs modified the relationship between 25(OH)D and hazard of relapse but did not reach significance | Prospective cohort study | McDonald | Western Pacific | 25(OH)D rs354033 (*ZNF767*) rs2119704 (*GALC*) rs908742 (*PRKCZ*) rs3783785 (*PRKCH*) | 141 RRMS cases | (Lin et al., 2014a) |
| | Alleles expressing 'non-responsive' *VDRE* motifs were associated with significantly reduced risk of MS suggesting vitamin-D regulation of *HLA-DR* expression is important | Case-control association | Poser/McDonald | Europe | *HLA-DRB1*04* *HLA-DRB1*07* *HLA-DRB1*09* | 466 cases 498 controls | (Nolan et al., 2012) |
| | *VDRE* motif variation does not contribute independently to increased MS risk | Case-control association | McDonald | Europe | *HLA-DRB1* alleles | 44 cases 112 controls homozygous for HLA-DRB1*1501 | (Cocco et al., 2012) |

### 6.5.4 Associations between vitamin D genes and vitamin D levels in MS (Table 6.21)

#### *The association between VDR variants and 25(OH)D levels in people with MS*

The association between serum 25(OH)D and *VDR* variants was explored in seven papers which focussed on rs731236 (*TaqI*), rs7975232 (*ApaI*), rs2228570 (*FokI*), and rs1544410 (*BsmI*). The findings for rs731236, rs7975232, and rs1544410 came from four papers. These studies, from European and Eastern Mediterranean countries, were all of a case-control design. They all agreed that rs731236, rs7975232, and rs1544410, are not associated with vitamin D levels in patients with MS (Al-Temaimi et al., 2015; Agnello et al., 2016; Yamout et al., 2016; Smolders et al., 2009a).

Agreement was not unanimous in the papers regarding rs2228570 and vitamin D levels in people with MS. One of the five studies that explored rs2228570, was a twin study, originating from The Americas. This study found no association between rs2228570 and vitamin D levels in MS (Orton et al., 2008). However, two of the remaining four studies, both case-control designs and from Europe, found that rs2228570 was significantly associated with higher vitamin D levels (Al-Temaimi et al., 2015), and higher summer vitamin D levels (Smolders et al., 2009b). The two remaining papers found associations between rs2228570 ff genotypes and vitamin D in MS. One study found that rs2228570 ff genotype was associated with significantly higher vitamin D than FF and Ff genotypes (Agnello et al., 2016). The other found an interaction between the ff genotype and dietary vitamin D intake which was associated with higher levels of vitamin D and with a reduced risk of MS (Simon et al., 2010b). Although the hypotheses tested are also slightly different, findings that rs2228570 may have an effect on vitamin D levels in people with MS are relatively consistent.

#### *The association between DBP genes and serum 25(OH)D levels in people with MS*

Only one paper explored DBP genes and vitamin D levels in people with MS. This study was cross-sectional, and attempted to replicate the effect of SNPs which

were associated with 25(OH)D in a GWAS of healthy European cohorts. The effects were replicated: significant associations were observed between SNPs rs7041 and rs2282679 and blood vitamin D levels in people with MS. Furthermore, the C allele of rs7041 and the T allele of rs2282679 produced an additive effect of a 4.4 nmol/L (95% CI 0.9-7.9) and 4.5 nmol/L (95% CI 0.6-8.4) increase in blood vitamin D respectively (Laursen et al., 2015). However, no correction for multiple testing was applied to the analyses, leading to an inflated possibility of type 1 errors. Further results from this study are discussed below.

### *The association between vitamin D metabolising genes and serum 25(OH)D levels in people with MS*

Four papers studied the effect of several metabolising genes on blood vitamin D levels in people with MS. Heterozygosity in *CYP27B1* was significantly associated with low calcitriol levels (Ramagopalan et al., 2011), and two other SNPs in the same gene were also significantly associated with vitamin D levels in MS and controls (Orton et al., 2008). *CYP2R1* genes were also associated with blood vitamin D in MS. *CYP2R1* encodes the enzyme vitamin D 25-hydroxylase, and is thought to be an important part of the process which converts cholecalciferol to calcidiol. In both *CYP2R1* rs2060793 and rs10741657 the effect of the A allele was found to be dominant. Additionally, the presence of the A allele in rs10741657 resulted in a 6.9 nmol/L (95% CI 3.3-10.4) increase in blood vitamin D in people with MS (Laursen et al., 2015).

However, another study found that a different SNP in *CYP2R1* was associated with lower blood vitamin D levels. Individuals who were heterozygous for the minor allele had significantly lower vitamin D levels (7.1 nmol/L (95% CI 2.12-12.08)) than those who were homozygous. Additionally, those who were homozygous for the major allele had vitamin D levels 12.4 nmol/L (95% CI 3.94-20.89) lower (Lin et al., 2014a). The variety of vitamin D metabolising SNPs and genes studied make it difficult to draw

conclusions regarding their involvement in blood vitamin D and MS, however there does appear to be a suggestion that vitamin D metabolising genes are involved with blood vitamin D levels in people with MS.

One study also looked at *NADSYN1*, which encodes an enzyme involved in the synthesis of cholesterol from 7-dehydrocholesterol. Although the two SNPs explored were significantly associated with vitamin D in a GWAS of a healthy cohort, the finding was not replicated in this MS study (Laursen et al., 2015).

### *The association between other genes and serum 25(OH)D levels in people with MS*

Other genes that were explored in relation to 25(OH)D levels in people with MS included two that are involved in cell adhesion (*VCAM1* and *CD6*), one involved in protein binding (*PLEK*), another in cell proliferation, growth and cell cycle progression (*RPS6KB1*), one with a role in mitochondrial ribosome assembly and stability (*MPV17L2*), and one protein kinase gene with multiple cellular functions including insulin signalling and endothelial cell regulation (*PRKCB*). Of these, the *VCAM*, *PLEK* and *RPS6KB1* SNPs were associated with significantly higher vitamin D levels in people with MS. A dose-response relationship was observed. People who were homozygous for the minor allele had significantly higher vitamin D levels compared to those homozygous for the common allele (Lin et al., 2014a). The other two SNPs, for *CD6* and *MPV17L2*, were associated with lower vitamin D levels, again exhibiting a dose-response relationship (Lin et al., 2014a). Additionally, a further paper by the same authors found that a SNP in *PRKCB* was associated with lower vitamin D levels, and showed a cumulative effect of decreasing vitamin D levels when it presents with *CYP2R1* (Lin et al., 2014b).

Table 6.21 Studies assessing the association between vitamin D genes and serum vitamin D in MS

| | Finding | Study design | Diagnostic criteria | Study region | Measures | Sample size | Authors |
|---|---|---|---|---|---|---|---|
| **To assess the association between *VDR* variants and serum 25(OH)D levels in patients with MS** | *TaqI* was not associated with vitamin D levels in people with MS | Case-control association | Neurologist-confirmed | Eastern Mediterranean | 25(OH)D rs731236 (*TaqI*) | 50 cases 50 controls | (Al-Temaimi et al., 2015) |
| | | | | Europe | 25(OH)D rs731236 (*TaqI*) | 104 cases 75 controls | (Agnello et al., 2016) |
| | | | McDonald | Eastern Mediterranean | 25(OH)D rs731236 (*TaqI*) | 50 cases 48 controls 51 controls with neurological disease | (Yamout et al., 2016) |
| | | | | Europe | 25(OH)D $1,25(OH)_2D$ rs731236 (*TaqI*) | 212 cases 289 controls | (Smolders et al., 2009a) |
| | *ApaI* was not associated with vitamin D levels in people with MS | Case-control association | Neurologist-confirmed | Eastern Mediterranean | 25(OH)D rs7975232 (*ApaI*) | 50 cases 50 controls | (Al-Temaimi et al., 2015) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | Europe | 25(OH)D<br>rs7975232<br>(*ApaI*) | 104 cases<br>75 controls | (Agnello et al., 2016) |
| | | | McDonald | Eastern Mediterranean | 25(OH)D<br>rs7975232<br>(*ApaI*) | 50 cases<br>48 controls<br>51 controls with neurological disease | (Yamout et al., 2016) |
| | | | | Europe | 25(OH)D<br>1,25(OH)$_2$D<br>rs7975232<br>(*ApaI*) | 212 cases<br>289 controls | (Smolders et al., 2009a) |
| | *FokI* was associated with higher vitamin D levels | Case-control association | Neurologist-confirmed | Eastern Mediterranean | 25(OH)D<br>rs2228570<br>(*FokI*) | 50 cases<br>50 controls | (Al-Temaimi et al., 2015) |
| | *FokI* was associated with higher summer vitamin D levels | Case-control association | McDonald | Europe | 25(OH)D<br>1,25(OH)$_2$D<br>rs2228570<br>(*FokI*) | 212 cases<br>289 controls | (Smolders et al., 2009b) |
| | *FokI* FF and Ff genotype carriers with MS had significantly lower 25(OH)D compared to ff carriers with MS | Case-control association | Neurologist-confirmed | Europe | 25(OH)D<br>rs2228570<br>(*FokI*) | 104 cases<br>75 controls | (Agnello et al., 2016) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | There was an interaction between dietary vitamin D and the ff genotype conferring a protective effect | Nested case-control | Poser | The Americas | FFQ<br>rs2228570 (*FokI*) | 214 cases<br>428 controls | (Simon et al., 2010b) |
| | There was no association between *FokI* SNP and 25(OH)D levels | Twin | Neurologist-confirmed | The Americas | 25(OH)D<br>rs2228570 (*FokI*) | 198 twin persons<br>(16 concordant pairs) | (Orton et al., 2008) |
| | *BsmI* was not associated with vitamin D levels in people with MS | Case-control association | Neurologist-confirmed | Eastern Mediterranean | 25(OH)D<br>rs1544410 (*BsmI*) | 50 cases<br>50 controls | (Al-Temaimi et al., 2015) |
| | | | | Europe | 25(OH)D<br>rs1544410 (*BsmI*) | 104 cases<br>75 controls | (Agnello et al., 2016) |
| | | | McDonald | Eastern Mediterranean | 25(OH)D<br>rs1544410 (*BsmI*) | 50 cases<br>48 controls<br>51 controls with neurological disease | (Yamout et al., 2016) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | Europe | 25(OH)D<br>1,25(OH)$_2$D<br>rs731236 (*TaqI*),<br>rs7975232 (*ApaI*) | 212 cases<br>289 controls | (Smolders et al., 2009a) |
| **To assess the association between *DBP* genes and serum 25(OH)D levels in patients with MS** | Significant associations were found between 25(OH)D and SNPs originally identified in GWAS of healthy cohort | Cross-sectional | McDonald | Europe | 25(OH)D<br>rs7041<br>rs2282679 | 1497 cases | (Laursen et al., 2015) |
| **To assess the association between vitamin D metabolising genes and serum 25(OH)D levels in patients with MS** | Heterozygosity in *CYP27B1* variant confers significant low calcitriol levels in MS | Whole exome sequencing of 1 MS case from each of 43 multicase families | Poser | The Americas | rs118204009 (*CYP27B1*) | 1 affected individual from each of 43 multicase families | (Ramagopalan et al., 2011) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Two SNPs were significantly associated with 25(OH)D levels in cases and controls | Twin | Neurologist-confirmed | The Americas | 25(OH)D<br>rs4646536 (*CYP27B1*)<br>rs703842 (*CYP27B1*) | 98 twin persons (16 concordant pairs) | (Orton et al., 2008) |
| | One SNP was significantly associated with lower 25(OH)D levels | Prospective cohort study | McDonald | Western Pacific | 25(OH)D<br>rs1993116 (*CYP2R1*) | 141 cases | (Lin et al., 2014b) |
| | Significant associations were found between 25(OH)D and SNPs identified in GWAS of healthy cohort | Cross-sectional | McDonald | Europe | 25(OH)D<br>rs10741657<br>rs2060793 (*CYP2R1*)<br>rs3829251<br>rs12785878 (*NADSYN1*) | 1497 cases | (Laursen et al., 2015) |
| **To assess the association between other genes and serum 25(OH)D levels in patients with MS** | Five SNPs were associated with 25(OH)D levels | Prospective cohort study | McDonald | Western Pacific | 25(OH)D<br>rs11581062 (*VCAM1*)<br>rs7595037 (*PLEK*)<br>rs180515 (*RPS6KB1*)<br>rs17824933 (*CD6*)<br>rs874628 (*MPV17L2*) | 141 cases | (Lin et al., 2014a) |

| | | | | | |
|---|---|---|---|---|---|
| One SNP was significantly associated with lower 25(OH)D levels | Prospective cohort study | McDonald | Western Pacific | 25(OH)D rs7404928 (*PRKCB*) | (Lin et al., 2014b) |

### 6.5.5 Associations between genes and UV exposure in MS (Table 6.22)

#### *The association between VDR variants and UV exposure in people with MS*

The association between *VDR* variants, UV exposure, and MS was assessed in two studies. Genotype ff[30875] was significantly protective against disability in cases stratified by sun exposure (median years' exposure/year of life) and sunbathing habits (never, versus rarely/occasional/frequent) (Mamutse et al., 2008). One further study from Tasmania found a significantly increased risk of MS among people who reported low childhood winter sun exposure and were GG compared to AA homozygous in the Cdx-2 *VDR* variant. A dose-response relationship was also observed, with heterozygous people having an intermediate risk. There was however no significant association with MS risk in the same gene in people who reported higher childhood sun exposure (Dickinson et al., 2009). These two papers present diverse findings, however both identified protective interactions between *VDR* variants and UV exposure on MS disability and risk, for which more research is needed for clarity.

#### *The association between other genes and UV exposure in people with MS*

Other genes that were explored in relation to UV exposure and MS were all variants of the melanocortin 1 receptor (*MC1R*) gene, which, among other things, can lead to an increased sensitivity to UV exposure. Three studies reported a significant increased risk of MS or worse disease outcome in those with *MC1R* variants (Partridge et al., 2004; Strange et al., 2010), one of which reported that the effect was most strong in women (Dwyer et al., 2008). The other study, a cross-sectional design, found that of the six variants studied, four variants were not associated with disability. However, the remaining two variants were significantly associated with greater disability. People with a history of childhood sunburn and Asp294His CG/GG genotypes had lower MSSS scores than those with childhood sunburn and CC genotypes, and there was no significant association between these variants, MSSS scores, and childhood weekend sun exposure (Strange et al., 2010). A further study found that increased childhood

summer sun exposure was protective against MS only in people who do not have red hair colour (RHC) variants of *MC1R* (Dwyer et al., 2008). Some of the *MC1R* variants are therefore associated with MS, and interact with UV exposure apparently mitigating MS risk in European and Tasmanian populations. However, as different hypotheses were addressed, there is again no replication of findings.

Table 6.22 Studies assessing the association between genes, UV exposure, and MS

| | Finding | Study design | Diagnostic criteria | Study region | Measures | Sample size | Authors |
|---|---|---|---|---|---|---|---|
| **To assess the association between *VDR* variants, UV exposure and MS** | *FokI* ff *VDR* gene polymorphism frequency was lower in cases with disease progression of EDSS >=6, and was protective against disability in cases stratified by UV exposure | Cross-sectional | Poser | Europe | AG[1229]<br>CG[3444]<br>GA[3944]<br>CC[20965]<br>CC[30056]<br>Ff[30875]<br>CT[48200]<br>Tt[65013] | 512 cases | (Mamutse et al., 2008) |
| | There was a significant interaction between winter childhood sun exposure, *Cdx-2* 'G' allele and increased MS risk. | Case-control association | Poser/MRI | Western Pacific | Cdx-2 A>G<br>rs2228570 (*FokI*) T>C<br>rs731236 (*TaqI*)C>T | 136 cases<br>272 controls | (Dickinson et al., 2009) |
| **To assess the association between other genes, UV exposure and MS** | *MC1R* receptor encoding alleles were associated with worse disease outcome | Case-control association | Poser | Europe | *MC1R* Asp84Glu | 419 cases<br>422 controls | (Partridge et al., 2004) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *MC1R* variants were associated with disability by MSSS | Cross-sectional | Poser | Europe | *MC1R*: rs1805006 (Asp84Glu) rs1805009 (Asp294His) | 525 cases | (Strange et al., 2010) |
| | *MCR1* receptor encoding alleles were associated with increased MS risk | Case-control association | Poser | Europe | *MC1R* Asp294His | 419 cases 422 controls | (Partridge et al., 2004) |
| | RHC variant of *MCR1* gene was significantly associated with MS, most strongly in women | Case-control association | Poser/MRI | Western Pacific | 25(OH)D Sun exposure *MC1R* Asp294His *MC1R* Arg151Cys *MC1R* Arg160Trp | 136 cases 272 controls | (Dwyer et al., 2008) |
| | Increased summer sun exposure at ages 6-10 was associated with reduced MS risk in those with no RHC variant but not in those with RHC variant genotypes | Case-control association | Poser/MRI | Western Pacific | 25(OH)D Sun exposure *MC1R* Asp294His *MC1R* Arg151Cys *MC1R* Arg160Trp | 136 cases 272 controls | (Dwyer et al., 2008) |

| | | | | | | |
|---|---|---|---|---|---|---|
| *MC1R* variants were not associated with disability by MSSS | Cross-sectional | Poser | Europe | *MC1R*: rs2228479 (Val92Met) rs1805007 (Arg151Cys) rs1805008 (Arg160Trp) rs1805005 (Val60Leu) | 525 cases | (Strange et al., 2010) |

### 6.5.6 Mendelian randomisation studies

Only one Mendelian Randomisation study was identified in the searches for this review (Mokry et al., 2015). This study sought to investigate whether genetically-lowered levels of blood vitamin D were associated with MS. Four SNPs associated with 25(OH)D were initially identified in a GWAS of the SUNLIGHT consortium. These SNPs comprised rs2282679 (DBP), rs6013897 (*CYP24A1*) and rs10741657 (*CYP27R1*) (vitamin D metabolism), and rs12785878 (DHCR7). Further testing using the Canadian Multicentre Osteoporosis Study demonstrated that 25(OH)D-decreasing alleles were significantly associated with lower levels of circulating 25(OH)D. The Mendelian randomisation study was then carried out using these SNPs and the Multiple Sclerosis Genetic Consortium Study.

Results showed that people who carry genes which lead to lower circulating vitamin D levels have a significantly increased risk of MS. Such findings provide promising evidence for a causal relationship between long-term vitamin D deficiency and MS.

### 6.5.7 Summary

We identified a large number of studies which researched genes involved in vitamin D, UV, and MS. Although we categorised the studies under various headings, very few themes emerged from the literature with regards to patterns of findings or replication of findings. To summarise, the following areas were explored in the literature:

1. **Identifying susceptibility loci in MS:** we found only one GWAS as a result of our searches, which identified gene *CYP27B1* as being a possible causal gene
2. **The role of genes in vitamin D and MS:** *VDR* variants rs731236 (*TaqI*), rs731236 (*ApaI*), rs2228570 (*FokI*), and rs7975232 (*BsmI*) were all explored in multiple papers with little consensus. Two possible replicated effects were

observed, firstly a positive association between rs731236 C alleles and MS in two world regions, and secondly no association between rs2228570 and MS in three world regions. There was no agreement for rs731236 or rs7975232 studies. Likewise, no associations were observed in two studies investigating DBP genes, and three replication attempts from two world regions failed to find associations between vitamin D metabolism genes and MS previously identified by GWAS

3. **The role of genes in vitamin D and MS risk, onset, and clinical course:** rs731236 (*TaqI*) showed possible associations with MS risk and progression when it interacts with *HLA-DRB1*1501*; however there was no other association with MS risk, onset, or clinical course. The other *VDR* variants likewise presented an ambiguous picture. Further possible interactions between *HLA-DRB1*1501* alleles with vitamin D metabolising genes may increase or reduce MS risk.
4. **Associations between vitamin D genes and serum vitamin D levels in people with MS:** four papers which were consistent in diagnostic criteria and from two world regions were in agreement that there was no association between blood vitamin D levels in people with MS and *VDR* variants rs731236 (*TaqI*), rs7975232 (*ApaI*), or rs1544410 (*BsmI*). Overall there was some evidence that rs2228570 (*FokI*) may have an effect on blood vitamin D levels in MS, but there was a general lack of consistency in the hypotheses tested. The role of DBP on blood vitamin D levels in MS was replicated in one paper from an original GWAS. Of the vitamin D metabolising gene, *CYP27B1* appears to have an effect on blood vitamin D in MS, while the roles of *CYP21R* and *NADSYN1* are not quite as clear.

5. **Associations between genes and UV exposure in people with MS:** there was some evidence to suggest that UV exposure may protect against MS risk and disability level in some individuals with particular genotypes. There was however no replication of these effects.
6. **Mendelian randomisation:** The Mendelian randomisation study used DBP SNPs, vitamin D metabolising genes, and genes involved in vitamin D synthesis as proxies for the environmental exposure of vitamin D deficiency. They found evidence between long-term, or lifetime, vitamin D deficiency, and MS risk. This design provides perhaps the best evidence for a causal association between vitamin D deficiency and MS risk which requires replication.

The literature regarding genes, vitamin D, UV, and MS, therefore adds up to a large, heterogeneous evidence base. Many of the studies identified through this scoping study comprise sample sizes that are too small to have the power to detect such small effect sizes, and as replication is considered to be the gold standard in confirming an effect, the literature is still largely lacking.

## 6.6 Discussion and Conclusion

### 6.6.1 Discussion

This scoping review was based upon a methodical and comprehensive search of the literature regarding MS, vitamin D, and UV exposure, from which we identified over one hundred relevant studies. To aggregate and present the findings in a meaningful way, we divided the studies into three broad categories and tabulated relevant data as both an overview of literature and to group findings for comparison. We then discussed the results in a narrative synthesis. This Discussion aims to integrate the main findings from the observational, intervention, and genetic study results to discuss the evidence for an association between vitamin D, UV exposure, and MS onset, pathology, and progression.

#### *6.7.1.1 The association between vitamin D, UV exposure, and MS*

Each category of observational, intervention, and genetic studies contained a large and heterogeneous body of work comprising multiple study designs, diagnosis methods, types of vitamin D, and from multiple world regions. No study was conducted in any African country; additionally, no intervention study, and only two each of genetic and observational studies, came from South-East Asia. This means that large areas globally are under-represented in the literature and therefore little understood. The vast majority of research came from Europe and the Americas (mainly North America), with the remainder from the Eastern Mediterranean, particularly Iran, and the Western Pacific, usually Australia.

The role of UV exposure in MS was considerably less-well researched in each category than vitamin D. The independent actions of vitamin D and UV exposure that have been noted in observational and animal studies, as well as the established importance of UV exposure on vitamin D synthesis (Lucas et al., 2011; Becklund et al., 2010; Wang et al., 2013; Wang et al., 2015), would suggest that UV exposure is a potentially valuable line of research. Additionally, large observational studies with quantitatively-measured UV exposure may help to ascertain how UV exposure may affect MS risk in addition to, and independent of, vitamin D. Furthermore, greater understanding about the actions of genes on exposure to UV radiation would be beneficial.

However, despite the literature lacking in these areas, a number of themes emerged with complementary and opposing findings. We summarised these findings at the end of each section, and review and collate the results here:

1) **Blood vitamin D:** observational studies have fairly consistently found lower vitamin D levels in people with MS compared to controls. Moreover, vitamin D

levels are often lower in people at the time of MS diagnosis compared to controls. However, as discussed, difficulties in obtaining accurate estimates of vitamin D that are representative of an individual's long-term vitamin D status, may impact the results of these observational studies and lead to some contradictory findings. From the genetic studies, blood vitamin D levels do not appear to be associated with *VDR* variants rs731236 (*TaqI*), rs7975232 (*ApaI*), or rs1544410 (*BsmI*), however rs2228570 (*FokI*) may be associated with significantly higher vitamin D levels in people with MS. DBP SNPs rs7041 and rs2282679 were also associated with higher vitamin D levels, although the vitamin D metabolising genes produced more heterogeneous results. The experimental literature unanimously found that vitamin D supplements were safe, well-tolerated, and effective at raising blood vitamin D levels in people with MS.

2) **MS risk and onset:** observational studies found that low vitamin D levels were not generally associated with MS risk or onset. However, a possible delay in conversion from ON to clinically definite MS was observed in one vitamin D therapy trial. Genetic studies identified possible interactions between a *VDR* SNP and *HLA-DRB1*1501* that may increase MS risk. The Mendelian randomisation study provides possibly the strongest support for an increased risk of MS with long-term vitamin D deficiency, which requires replication. Evidence from a genetic study found that UV exposure interacting with particular genotypes may be protective against MS. However, as these findings have not yet been replicated they need to be interpreted with extreme caution.
3) **MS disease activity:** A number of observational studies found that low vitamin D levels were associated with greater disease activity, were lower in people with RRMS, and were significantly lower in people who were experiencing a

relapse compared to those in remission. UV exposure may also have positive effects on disease activity although this association has been identified only through cross-sectional studies. The experimental evidence showed some potentially positive effects of vitamin D therapy in reducing disease activity, however the different methods used for measuring disease activity and in vitamin D dosage quantities make the studies difficult to compare. Unreplicated evidence from a genetic study, again therefore requiring extreme caution in interpretation, found that UV exposure interacting with particular genotypes may be protective against worsening MS disability.

4) **MS subtype and gender-specific observations:** Observational studies showed that increased sun exposure and vitamin D intake in childhood and adolescence may be protective against MS, however longitudinal evidence suggests that there is no effect of early vitamin D intake on later life MS risk. Nonetheless, two longitudinal studies found that low vitamin D levels were associated with paediatric-onset MS and the relapse rate in paediatric MS. Greater MS risk and MS progression were noted in women only, and women were found to have generally low vitamin D levels. Women were also found to have low levels of sun exposure and low vitamin D intake, however supplementation appeared to be protective against MS in women in a longitudinal study.

These themes show that there are patterns within the literature: many similar hypotheses have been tested and exploratory analyses conducted in different datasets. There is clear evidence of an association between MS and vitamin D, and MS and UV exposure.

### 6.6.2 Limitations

There are several limitations to this study. Firstly, a scoping study is an iterative process which is shaped according to the literature produced by the searches. After the first search, we refined the inclusion criteria to eliminate animal models, as the quantity of literature identified was unfeasible to adequately review given the amount of time and word count available.

Our inclusion criteria also originally specified papers that defined MS according to the Poser or McDonald criteria only; however following searches we decided to accept all papers, regardless of MS diagnosis method, that also met the other inclusion criteria. This is because it seemed important to understand how MS was being defined across the literature, and to thereby identify possible difficulties with comparison. We also broadened our original vitamin D inclusion terms to all types of vitamin D that were identified by searches, including vitamin D analogues. In genetic studies we included all articles involving vitamin D-related genes that met other inclusion criteria. In so doing, we cast a wide net to try to identify as much of the relevant literature as we could.

Secondly, the field of genetics has evolved very quickly. This means that the methods used in some of the genetic studies included herein are today considered outdated; the generally small sample sizes in older studies are also problematic as the power to detect associations – particularly for rare variants – will be too low. Furthermore, by only including studies that have been carried out in MS patients, and that explicitly mention vitamin D or UV exposure, it is likely that we have missed some relevant information from much larger, and more recent studies. Such studies include a recent International Multiple Sclerosis Genetics Consortium GWAS, which identified 48 novel, and 49 known, susceptibility variants for MS (International Multiple Sclerosis Genetics Consortium, 2013). This GWAS found that some of the genes studied in

publications included in this scoping review were significantly associated with MS, including *VCAM1*, *PLEK*, *GALC*, *CD40*, *CYP24A1,* and *CD6*, however the only SNP that was the same both in this scoping review and in the GWAS was rs2248359 in *CYP24A1*. This GWAS includes almost 15,000 MS cases and 25,000 controls, and therefore provides stronger evidence than any of the studies included herewith. That none of the other SNPs identified by publications included in this review appear in this GWAS means that the evidence against any real associations is fairly strong. Other GWAS of vitamin D in non-MS populations may also have yielded useful information regarding genes that affect vitamin D. The SUNLIGHT consortium GWAS, involving a sample size of upwards of 30,000 individuals, confirmed previous associations between circulating vitamin D levels and rs2282679 (*GC*), rs10741657 (*CYP2R1*), and rs6013897 (*CYP24A1*). They also identified a novel association with rs12785878 (*DHCR7*) (Wang et al., 2010). All these susceptibility variants have since been confirmed in a further study in a UK population which found an association with type 1 diabetes (Cooper et al., 2011), and furthermore, have all been found to be associated with low circulating vitamin D and an increased risk of MS in the Mendelian randomisation study discussed earlier (Mokry et al., 2015).

Thirdly, our searches resulted in the identification of a wide-ranging and large literature which concentrated exclusively on individually-measured vitamin D, vitamin D genes, and UV exposure or related genes in people with MS. Searching the literature for specific comorbidities, such as bone mineral conditions, could have yielded further studies with individual vitamin D measures and possibly also UV exposure data. There were similarly other lines of research that we could have explored, including cellular effects of vitamin D deficiency in MS, and also possible interactions between vitamin D deficiency and medication. There is some evidence to suggest that interferon β, used in the treatment of MS, is only effective when vitamin D levels are high; treatment with

interferon β when vitamin D levels are low appears to increase risk of relapse (Stewart et al., 2012). This may explain some of the findings regarding low vitamin D and risk of relapse and may have contributed further understanding to our scoping review. Additionally, focussing further on the known genetic risk of *HLA-DRB1*1501* and how it may interact with vitamin D deficiency could have broadened the review to more deeply understand further mechanisms in the way that a genetic risk factor and widespread environmental risk factors interact. However, we were unable to follow all the literature trails, but there is scope for further studies to explore in detail these other areas.

Finally, vitamin D and UV exposure in MS are fast-moving areas of research, and this study is therefore limited by the time at which the searches were conducted. This review is thus an overview of the literature on the search day, but it should be noted that several papers have since been published which would have contributed to this scoping study. Of note, a second Mendelian randomisation study was published which explored three SNPS, one of which had been explored in the previous Mendelian randomisation study and two of which were different. The findings were however complementary to the effects observed in the first study, thereby lending further support to a possible causal link between vitamin D deficiency and MS risk (Rhead et al., 2016). Additionally, a third Mendelian randomisation study was published which looked into evidence for a causal relationship between low vitamin D and high BMI in paediatric-onset MS (Gianfrancesco et al., 2017). It found that children with a genetic risk score that indicates higher circulating vitamin D levels had a reduced risk of paediatric-onset MS (Gianfrancesco et al., 2017). Vitamin D deficiency was variously found to be associated with, and not associated with, paediatric-onset MS in the observational literature, and therefore this study adds considerable evidence to the existing studies that vitamin D may be a significant risk factor. Finally, another cross-

sectional study found an effect of season and relapse, whereby the strong seasonal variation of circulating vitamin D was negatively correlated with the rate of relapse (Hartl et al., 2017).

### 6.6.3 Conclusion

This scoping study aimed to provide an overview of the existing literature examining vitamin D and UV radiation in the context of MS onset, pathology, and progression. To be transparent about our methods, we followed Arksey and O'Malley's recommendations alongside those of Levac.

It is clear that a large amount of work has been undertaken and is ongoing, and has resulted in a very heterogeneous literature. The observational literature was wide-ranging, incorporating multiple world regions and study designs, and clearly showed that low vitamin D levels are associated with MS. However, without excluding reverse causation, the role of vitamin D deficiency in MS risk and progression is still unclear. The literature is also still ambiguous regarding the roles of UV exposure, and dietary vitamin D intake and supplementation, particularly at certain times of life, and their potential impact on MS risk, onset, and progression.

As highlighted by the systematic reviews discussed in the introduction, the intervention literature has scope for further investigations into the role of vitamin D in MS. New RCTs exploring both the possible therapeutic role of vitamin D, and also its possible protective effect in preventing or delaying onset, would be beneficial. Such RCTs should however include large enough doses of vitamin D, and be of a long enough duration, to produce and observe any beneficial effects.

Finally, the field of genetics is producing a vast amount of literature in this area of research, however there appear to have been few concerted attempts at replication. The amount of literature, so much of which is disparate and contradictory, has not

provided much clarity regarding the roles of different genes and variants involved with vitamin D, UV exposure, and MS. There are, therefore, still multiple questions that need answering, and scope for meta-analyses of GWAS which would provide clarity.

However, there is clearly an association between MS, vitamin D, and UV exposure. To further understand the roles of vitamin D and UV exposure as potential MS risk factors in the Northern Isles, the following two chapters present the primary studies undertaken for this thesis. Chapter 7 concentrates on vitamin D in Orkney, while Chapter 8 explores UV exposure in Shetland.

# CHAPTER 7. FARMING, FOREIGN HOLIDAYS AND VITAMIN D IN ORKNEY

## 7.1 Introduction

In earlier chapters I introduced Orkney, detailing its high-latitude geographical location, climate, and high prevalence of MS. The heritability analysis suggested that, as elsewhere, a genetic predisposition is necessary but other risk factors must also be present for disease to manifest. In Chapter 6 I reviewed the literature regarding vitamin D, UV, and MS, and showed that vitamin D deficiency is an important risk factor for MS with an increasing evidence base. Vitamin D deficiency is therefore a pertinent risk factor for investigation in Orkney.

The body of this chapter, from section 7.2 to 7.2.5, consists of my paper, *Farming, foreign holidays, and vitamin D in Orkney*, which was published in May 2016 (Weiss et al., 2016). The published version can be found at http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0155633, and is presented in Appendix D. As outlined in the paper, James F Wilson, Ruth McQuillan, and I, conceived and designed the study. Stephanie Read gave me statistical advice regarding how to carry out the multiple imputation for missing data, and Lina Zgaga supplied the R script to convert crude vitamin D into May-adjusted vitamin D thus removing seasonal effects, and which had been previously used in a paper involving vitamin D measurements (Zgaga et al., 2011). I ran all analyses. Data were contributed by Harry Campbell and Malcolm G Dunlop (Scottish Colorectal Cancer Study (SOCCS)) and James F Wilson (ORCADES). Blood from ORCADES was analysed for vitamin D levels by a Glasgow laboratory in 2011, which had previously analysed samples for SOCCS using the same methods. I wrote the majority of the paper, into which I incorporated comments from all co-authors and from two reviewers, one of whom

remained anonymous and the other of whom chose to sign his review and was William Grant of the Sunlight, Nutrition, and Health Centre (SUNARC).

The paper begins with an introduction setting the research into context, before moving into the methods, results, a discussion, and conclusion. However, this chapter will be rounded with a brief conclusion (section 7.3) to place the paper into the overall context of this thesis.

## 7.2 Farming, foreign holidays and vitamin D in Orkney

### 7.2.1 Introduction

Multiple sclerosis is a chronic, complex disease with genetic, environmental and behavioural factors implicated in its aetiology (Dyment et al., 2004). Greater distance from the equator is associated with increasing MS prevalence (Simpson S. Jr. et al., 2011); increasing latitude is also noted for weaker ultraviolet B (UVB) radiation which inhibits cutaneous production of vitamin D (Webb et al., 1988). As such, one environmental risk factor is thought to be vitamin D deficiency, however, vitamin D is also a marker for exposure to UV radiation, the benefits of which may be independent of vitamin D production (Becklund et al., 2010; Wang et al., 2013; Lucas et al., 2011). A variety of factors hinder or prevent UVB from reaching the earth's surface, including latitude and weather (Holick, 2008), or from initiating cutaneous vitamin D synthesis, such as sun protection creams and clothing cover.

Although a recent systematic review of vitamin D status worldwide found that vitamin D concentrations do not appear to be dependent upon latitude (Hilger et al., 2014), exposure to ultraviolet B (UVB) radiation from sunshine is the most potent source of vitamin D for humans (Holick, 2004a). In the United States, a latitudinal relationship exists between wintertime vitamin D and wintertime UV doses (Grant and Holick, 2005), likely resulting from the few days in which vitamin D can be produced at

such latitudes (Webb et al., 1988; Grant and Holick, 2005). This latitudinal relationship further reflects the latitudinal MS prevalence distribution in the US (Grant and Holick, 2005). Additionally, a significant relationship between regional UVB radiation and MS prevalence has been noted in France in the French farming population and their families (Orton et al., 2011). Increasing MS prevalence was associated with decreasing ambient UVB; the trend was additionally found to be stronger in both wintertime and in women (Orton et al., 2011). A similar relationship between decreasing UV and increasing prevalence has been identified in Newfoundland (Sloka et al., 2008) and Australia (van der Mei et al., 2001).

Controversy remains regarding the role of vitamin D in chronic conditions; whilst deficiency may have a causal role in the aetiology of some diseases it may also simply be a biomarker for ill health. A body of evidence is however accumulating, suggesting a causal role for vitamin D in MS risk, pathology and progression (Ascherio et al., 2014; Munger et al., 2006; Mokry et al., 2015). A recent Mendelian randomisation study found genetically lowered 25-hydroxyvitamin D (25(OH)D) level by one standard deviation in log-transformed 25(OH)D was associated with a two-fold increased risk of MS (Mokry et al., 2015). Interactions between vitamin D and the major genetic risk factor, HLA-DRB1*1501, have been identified (Ramagopalan et al., 2009b), and several rare variants conferring MS risk have been found in the *CYP27B1* gene, which encodes an enzyme which catalyses the conversion of 25(OH)D to the bioactive form (Ramagopalan et al., 2011). Further, early exposure to vitamin D in-utero and in neonatal mice led to optimal numbers of invariant natural killer T cells (Yu and Cantorna, 2011), deficiency of which are observed in MS patients (van der Vliet et al., 2001; Yu and Cantorna, 2011). Alongside the month-of-birth effect, where children born after a winter gestation are at higher risk of MS (Dobson et al., 2012), the role of

adequate in-utero vitamin D increasingly appears to be critical for future autoimmunity.

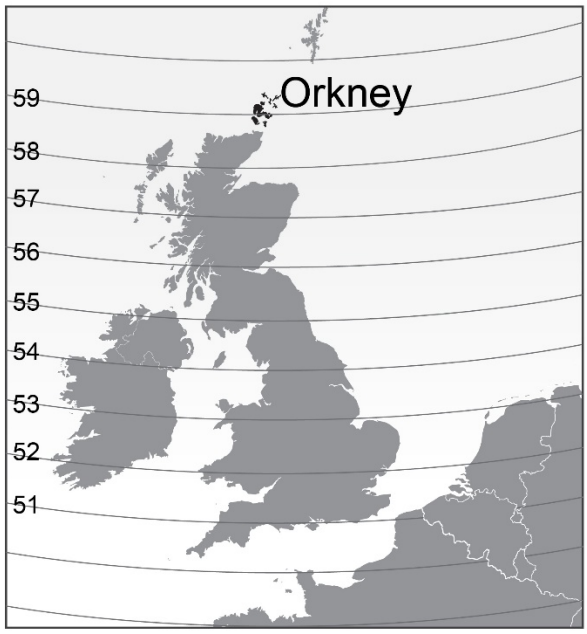
The beneficial role of UV exposure independent of vitamin D production has been supported in animal studies, using experimental autoimmune encephalomyelitis (EAE) as the model for MS. Continuous treatment with UVB was found to suppress clinical signs of EAE which, although leading to slight elevations in serum $25(OH)D_3$, were insufficient to cause disease suppression by vitamin D (Becklund et al., 2010). Furthermore, suppression of EAE was found to occur upon irradiation of mice to narrow-band UV light, with a wavelength of between 300 and 315 nm and a peak of effectiveness at 311 nm. As vitamin D requires a wavelength between 270 and 300 nm, optimally between 295 and 300 nm to initiate cutaneous synthesis, the narrow band UV supressing EAE had no effect on 25(OH)D levels (Wang et al., 2013), strongly suggesting a role of UV exposure independent of vitamin D. In MS, an Australian multi-centre case-control study found higher sun exposure and higher vitamin D levels to be independently associated with lower risk for first demyelinating events (Lucas et al., 2011).

Scotland, between latitudes $54^{o}$ and $60^{o}$ north, has inadequate strength of sunshine between October and March for vitamin D synthesis (Webb and Engelsen, 2006); a cloudy climate year-round further leads to widespread vitamin D deficiency (Rhodes et al., 2010) strongly indicating limited UV exposure in the population. The protective effect of supplementation and sunny holidays on 25(OH)D in Aberdeen, a Scottish city at $57^{o}$ north, has previously been noted in a study of postmenopausal women (Mavroeidi et al., 2013). Orkney, an isolated archipelago ten to sixty miles from the north coast of Scotland, is an area of exceptionally high MS prevalence (Visser et al., 2012). Seventeen of the 70 islands are inhabited with a predominantly rural population

totalling 21,349 at the 2011 census. The 2011 census also revealed an ongoing agricultural tradition with 10% of the workforce employed in agriculture or fishing.

As an independent risk factor, or as a marker of UV exposure, it is important to understand the determinants of plasma vitamin D in the context of MS and other diseases of public health importance. In this study we aimed to describe vitamin D levels in Orkney (Figure 7.1). This involved identifying the prevalence of vitamin D deficiency in Orkney compared to the Scottish mainland, and establishing the determinants of circulating plasma 25-hydroxyvitamin D (25(OH)D) in Orkney.

Figure 7.1 Map of Orkney in relation to the Scottish mainland and north-west periphery of Europe, with 57th and 59th degrees of latitude




### 7.2.2 Materials and Methods

#### *7.2.2.1 Study populations*

The study population comprised Orkney Complex Disease Study (ORCADES) participants, recruited from 2005 to 2011, and Scottish Colorectal Cancer Study (SOCCS) controls, which ran from 1999 to 2006. Both these studies have been described in detail elsewhere (McQuillan et al., 2008; Tenesa et al., 2008). Briefly,

ORCADES, a cross-sectional genetic epidemiology study concerned with identifying genetic factors that influence complex disease, comprised 2078 participants with at least one Orcadian grandparent. SOCCS, a case-control study of colorectal cancer in Scotland, included 2235 adult controls without colorectal cancer, identified from the Community Health Index in Scotland as being aged within 5 years their matched case, of the same sex and living in the same area. All participants provided written, informed consent prior to participation. Both studies have ethical approval from NHS Orkney, NHS Grampian or NHS Lothian.

#### *7.2.2.2 25-hydroxyvitamin D measurement*

Fasting blood samples were drawn from ORCADES participants using the Sarstedt Monovette system. Samples were processed and transferred for storage at -$40^0$ C, and later -$80^0$ C, until analysis. Blood was drawn from all SOCCS participants, processed and transferred for storage at -$80^0$ C. Both studies ran over multiple years, and therefore measures per month comprise blood drawn in multiple Januarys, multiple Februarys and so on.

Vitamin D status is determined by measuring circulating 25(OH)D which is generally considered the best indicator of vitamin D status (Seamans and Cashman, 2009). Both 25(OH)$D_2$ and 25(OH)$D_3$ were measured using the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The total of the two measures was taken for total circulating 25(OH)D, however most samples from both studies contained no 25(OH)$D_2$. The lower limit of detection using LC-MS/MS was 10 nmol/L. All samples were measured in the same laboratory following standard protocols; quality control procedures were performed according to current best evidence for 25(OH)D measurement in population studies (Wallace et al., 2010).

A range of cut-offs to define sufficiency and deficiency have been proposed, however in line with other recent studies we considered circulating 25(OH)D of 50 nmol/L or over to be sufficient (Ross et al., 2011), 25 to 50 nmol/L to be at risk of deficiency or insufficient, and deficiency to be less than 25 nmol/L (Pearce and Cheetham, 2010). We additionally explored those at the lower end of deficiency, where we considered circulating 25(OH)D below 12.5 nmol/L to be severely deficient (Zgaga et al., 2011).

#### *7.2.2.3 Lifestyle factors*

ORCADES participants attended clinics where several biometric measures were recorded. Each participant also completed a medical and lifestyle questionnaire from which vitamin D intake, physical activity (PA) and socioeconomic status (SES) were derived.

BMI was calculated as $kg/m^2$ and treated as a continuous variable. Height was taken without shoes and weight wearing only light clothing. Based on questionnaire data we derived a variable encompassing work and leisure time PA throughout the year. Participants classified leisure activity as either 1) light (mostly sitting, light housework) or 2) moderate exercise; and likewise work activity as 1) mostly sitting, 2) mostly standing, 3) manual work or 4) heavy manual work. Dietary vitamin D intake was estimated from two self-administered food frequency questionnaires, the cardiovascular disease questionnaire (CVDQ) and bone density questionnaire (BDQ). The CVDQ was treated as the primary source due to the higher response rate, however information contained in the BDQ that was not present in the CVDQ was merged to create the most comprehensive variable possible. Further, a research nurse-administered drug questionnaire sought information about medications and dietary supplements. Participants also described their frequency of taking holidays within or outside the UK (never, less than once a year, once a year, more than once a year).

Although the Scottish Index for Multiple Deprivation (SIMD) is available for Orkney, the scattered and heterogeneous population means that concentrations of poverty or affluence are difficult to identify; moreover neighbouring islands are grouped together in units thus there is little discrimination (Scottish Index of Mulitple Deprivation, 2012). Principal Components Analysis (PCA) is a statistical technique to reduce a number of variables into a few independent dimensions reflecting the underlying patterns in the data, and was used here to construct SES indices (Vyas and Kumaranayake, 2006). To establish a variable that differentiates between individuals, three SES variables were thereby derived from 10 questionnaire items with significant loadings in PCA (S1 Table). Additionally, we applied an occupational prestige score to questionnaire occupation information which was then included in the PCA (Nakao and Treas, 1990). Holidays, car age and council tax band loaded significantly onto the first component; housing tenure, length of car ownership and highest qualification loaded significantly onto the second component, and job prestige score, years in education and supervisory role at work loaded significantly onto the third. This third component captures "non-traditional" lifestyles reflecting managerial, administrative and professional positions in contrast to traditional agricultural work. Time outside in summer was summed from participants' estimates of average time spent without a roof covering on summer work and leisure days. Data from SOCCS included age, sex, month of blood sample and 25(OH)D measurement.

#### *7.2.2.4 Statistical analyses*

We matched the ORCADES and SOCCS datasets on age to within two years (Table 7.1) to remove any differences arising from age structures. Matching was carried out blind excepting dataset of origin and age. Because of the large effect of season on vitamin D levels, we standardised 25(OH)D measurements to the month of May; values obtained thereby represent those that would be expected if every sample were drawn

in May (Zgaga et al., 2011). The mean of monthly means in the mainland Scottish data is 34.4 nmol/L; in the Orcadian data the mean of monthly means is 37.7 nmol/L. The May means are 33.8 nmol/L and 35.5 nmol/L, respectively. We used May-standardised measurements for all analyses concerned with determinants of vitamin D, and also to compare Orkney and mainland Scotland in deficiency levels. For analyses concerned with vitamin D and time of year we used crude 25(OH)D measures. Data are presented as mean (standard deviation).

Table 7.1 Distribution of age and crude vitamin D in age-matched Orkney and mainland Scotland datasets. The mainland dataset excludes people from above the 57th degree of latitude

| | **Orkney No (%)** | **Mean 25OHD (nmol/L)** | **Mainland No (%)** | **Mean 25OHD (nmol/L)** |
|---|---|---|---|---|
| Participants | 1453 | 36.2 | 1453 | 35.4 |
| < 40 | 46 (3.17) | 26.8 | 46 (3.17) | 36.5 |
| 40 – 49 | 263 (18.1) | 33.4 | 263 (18.1) | 39.5 |
| 50 – 59 | 399 (27.5) | 35.7 | 400 (27.5) | 38.7 |
| 60 – 69 | 466 (32.1) | 37.9 | 464 (31.9) | 33.3 |
| 70 + | 279 (19.2) | 39.5 | 280 (19.3) | 30.4 |
| Sex (male) | 590 (40.6) | 37.2 | 794 (54.6) | 35.4 |
| Sex (female) | 863 (59.4) | 35.6 | 659 (45.4) | 35.4 |

We plotted crude 25(OH)D by location as a density plot and by month, and compared using a t-test. We compared vitamin D by age group using t-tests. To compare levels of deficiency in Orkney and mainland Scotland, we divided participants into groups of deficiency and plotted May-adjusted vitamin D for each deficiency group and location and tested for differences using chi-square tests.

For determinants of May-adjusted 25(OH)D in Orkney, we ran a series of bivariable models of May-adjusted 25(OH)D against environmental and demographic variables of interest. Those that were significant were put into a multivariable model.

These significant variables comprised BMI, age at venepuncture, foreign holidays, PA, SES, dietary vitamin D and working status. Sex was also a covariate. A large percentage of missing data (S2 Table) was imputed using Multiple Imputation of Chained Equations (MICE)(van Buuren and Goothuis-Oudshoorn, 2011) after excluding 28 individuals with missing outcome data. We ran 68 cycles of 100 imputations and pooled the results in a linear regression model. We ran the same model using complete cases only. Statistical tests were two-sided with $p<0.05$ taken as significant. Finally, we applied a one-way ANOVA to compare mean May-adjusted 25(OH)D across the three groups of participants that we identified as a result of our analyses.

We assessed homoscedasticity by inspection of a QQ plot, and distribution of residuals using a histogram with superimposed normal curve. Independence was checked using the Durbin Watson statistic, multicollinearity and outliers using the VIF statistic and Cook's distance, respectively. All analyses were conducted using R software version 3.2.0 (R Core Team, 2015).

### 7.2.3 Results

For this study, 64 individuals were excluded from ORCADES who were not resident in Orkney, 10 who had MS, as well as 8 duplicate measures. Characteristics of ORCADES participants are presented in Table 7.2. Twenty-three people were excluded from the Scottish Colorectal Cancer Study who lived above the 57$^{th}$ degree of latitude. For comparison analyses, data were age-matched giving a final count of 1453 people in each dataset.

Table 7.2 Characteristics of ORCADES Study participants (n=1972)

| Characteristic | No or mean | SD | % or range |
|---|---|---|---|
| Age at venepuncture (years) | 53.4 | 15.3 | 16.5 – 100.2 |
| Sex | | | |
| Female | 1191 | - | 60.4 |
| Male | 781 | - | 39.6 |
| Body Mass Index (kg/m$^2$) | 27.7 | 4.9 | 16.9 – 53.9 |
| Vitamin D intake (μg) | 4.4 | 3.1 | 0.00 – 34.1 |
| Physical activity* | 5.1 | 1.2 | 3.0 – 8.0 |
| Summer minutes outdoors | 223 | 142 | 4.8 – 900 |
| Working | 1367 | - | 69.3 |
| Retired | 547 | - | 27.7 |
| Holidays outside the UK | | | |
| < once a year | 1472 | - | 74.6 |
| Once a year | 329 | - | 16.7 |
| > once a year | 105 | - | 5.3 |
| Years in education | 16 | 1.2 | 1.0 – 23 |
| Qualification level | | | |
| O & standard grades, CSE | 275 | - | 13.9 |
| Highers, A levels | 787 | - | 39.9 |
| Certificates/diplomas | 739 | - | 37.5 |
| Bachelor/Master degree | 88 | - | 4.5 |
| Doctorate | 13 | - | 0.7 |

* Physical activity scored from 1 (mostly sitting; inactive) to 4 (heavy manual labour; active) across different domains within work and leisure. Each score is the sum of answers creating an individual value for each participant.

Using age-matched data we compared mean 25(OH)D in Orkney to mainland Scotland (Figure 7.2). Orkney had significantly higher crude 25(OH)D than mainland Scotland (Orkney 35.3 (18.01), Mainland 31.7 (21.18), $t(2800)$=-4. 93, p=8.5 x $10^{-7}$). Mean 25(OH)D was higher in Orkney for every month except August when the mainland peaks at ~50 nmol/L. The distribution of vitamin D levels is shifted to the right in Orkney (Figure 7.3). We compared vitamin D in Orkney and mainland Scotland

by age group (Table 7.3). Results for each age group are significantly different, however in the under 40s, 40 to 49 and 50 to 59 age groups, mainland Scotland has higher vitamin D, whilst in the 60 to 69 and over 70s age groups, Orkney has significantly higher vitamin D. Comparing Orkney with the mainland by deficiency group, we found that more people in Orkney had insufficient vitamin D, $\chi^2$ (1, N=2863)=30.3, p=3.8 x $10^{-8}$; however, Orkney had significantly fewer people with circulating 25(OH)D of <12.5nmol/L (severely deficient) compared with the mainland, $\chi^2$ (1, N=2863)=64.3, p=1.1 x $10^{-15}$ (Figure 7.4).

Table 7.3 Comparison of vitamin D in Orkney and mainland Scotland by age group

| | **N = Orkney; Scotland** | **Orkney mean crude 25(OH)D** | **Mainland mean crude 25(OH)D** | **t-test** | **p-value** |
|---|---|---|---|---|---|
| < 40 | 46; 46 | 26.8 | 36.5 | -2.67 | 0.009 |
| 40 – 49 | 263; 263 | 33.4 | 39.5 | -3.13 | 0.002 |
| 50 – 59 | 399; 400 | 35.7 | 38.7 | -1.97 | 0.049 |
| 60 – 69 | 466; 464 | 37.95 | 33.28 | 3.59 | 0.0004 |
| 70+ | 279; 280 | 38.51 | 30.39 | 4.84 | $1.6x10^{-6}$ |

Figure 7. 2 Mean crude vitamin D concentration (nmol/L) per month by location, using age-matched data. Orkney's mean vitamin D is higher than the mainland for every month except August and November. Each study ran over consecutive years and measurements taken in the same month each year were pooled.

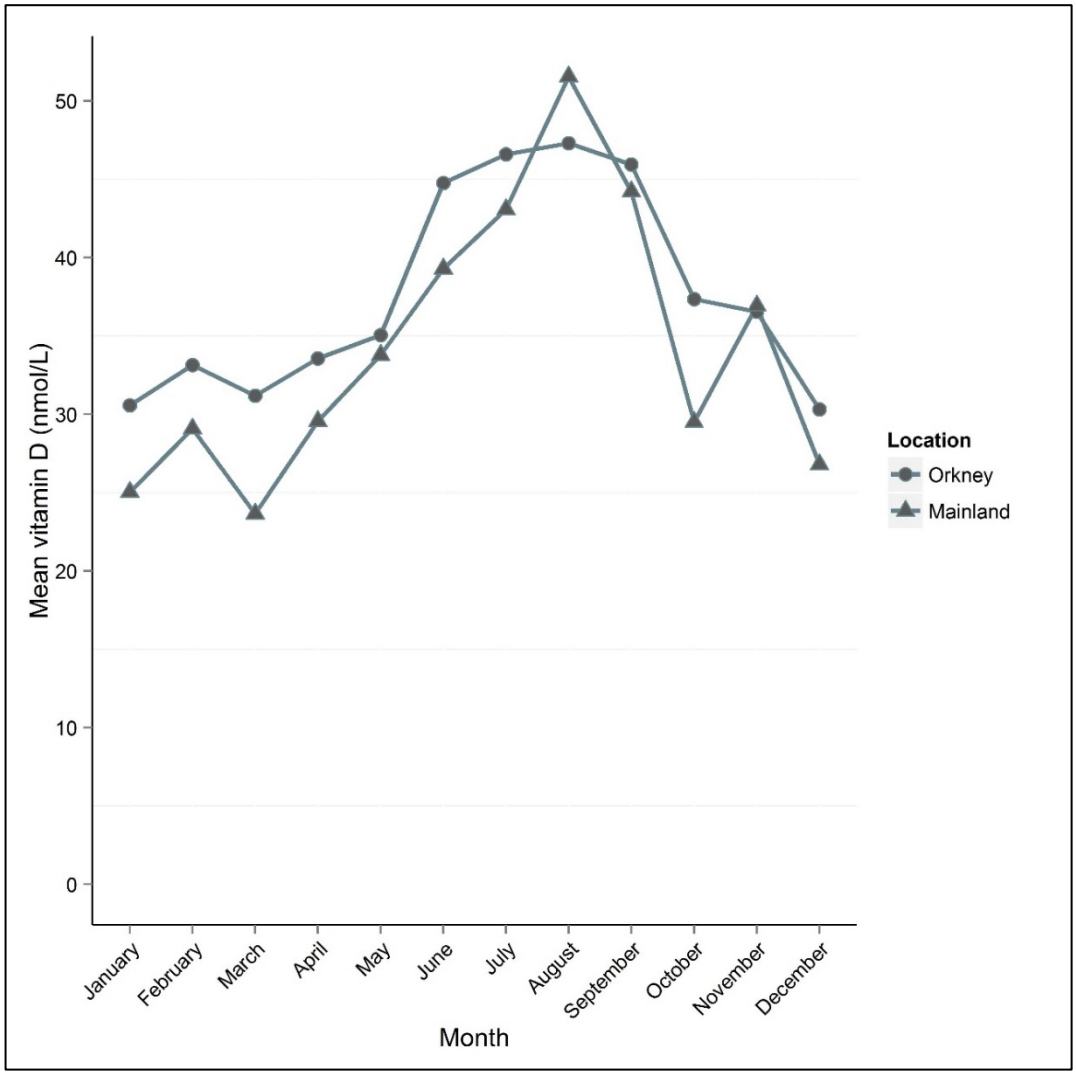


Figure 7. 3 Comparison of May-adjusted vitamin D distribution in Orkney and mainland Scotland using age-matched data. The distribution for Orkney is to the right of the distribution for the mainland, reflecting the lower prevalence of severe deficiency, and peaks higher.

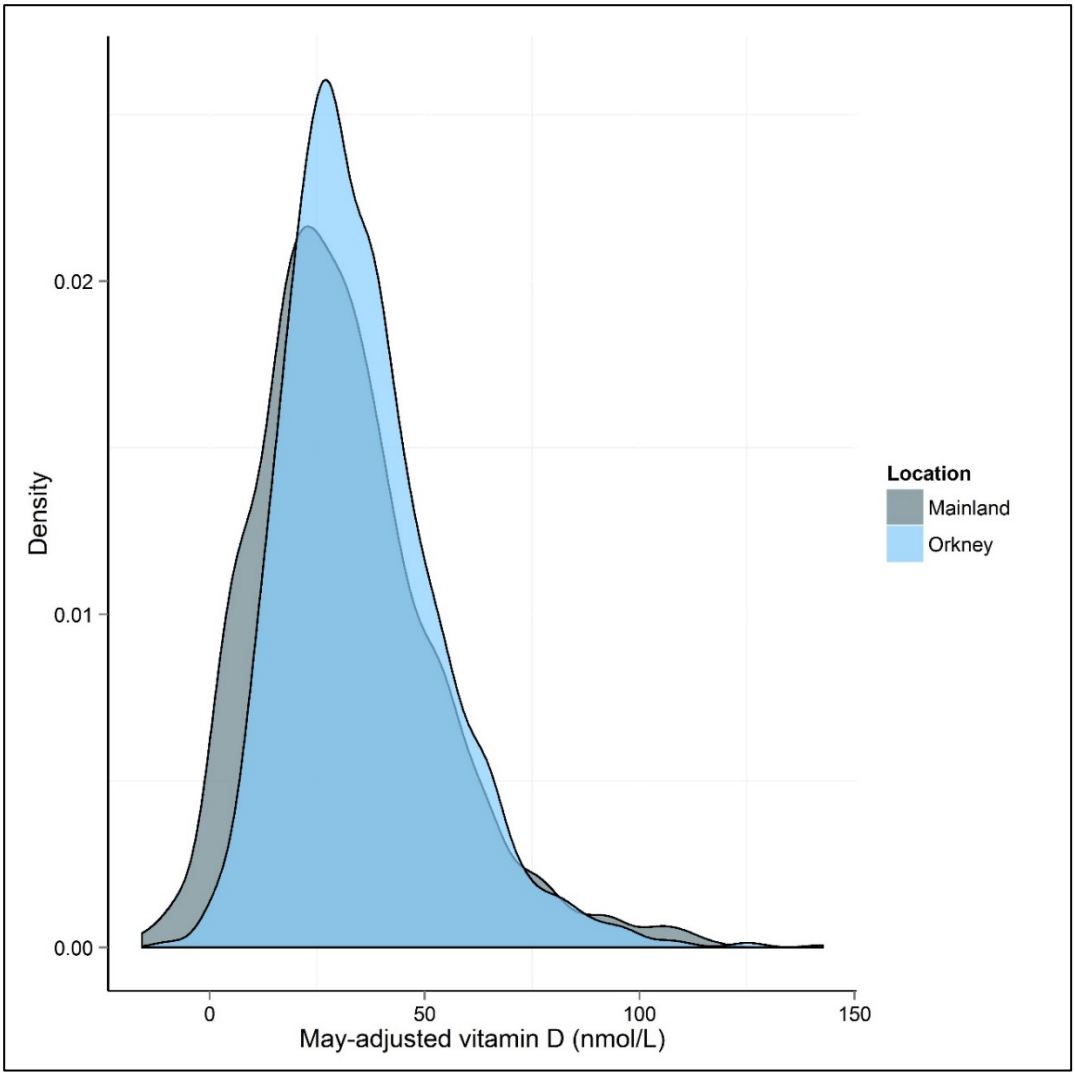
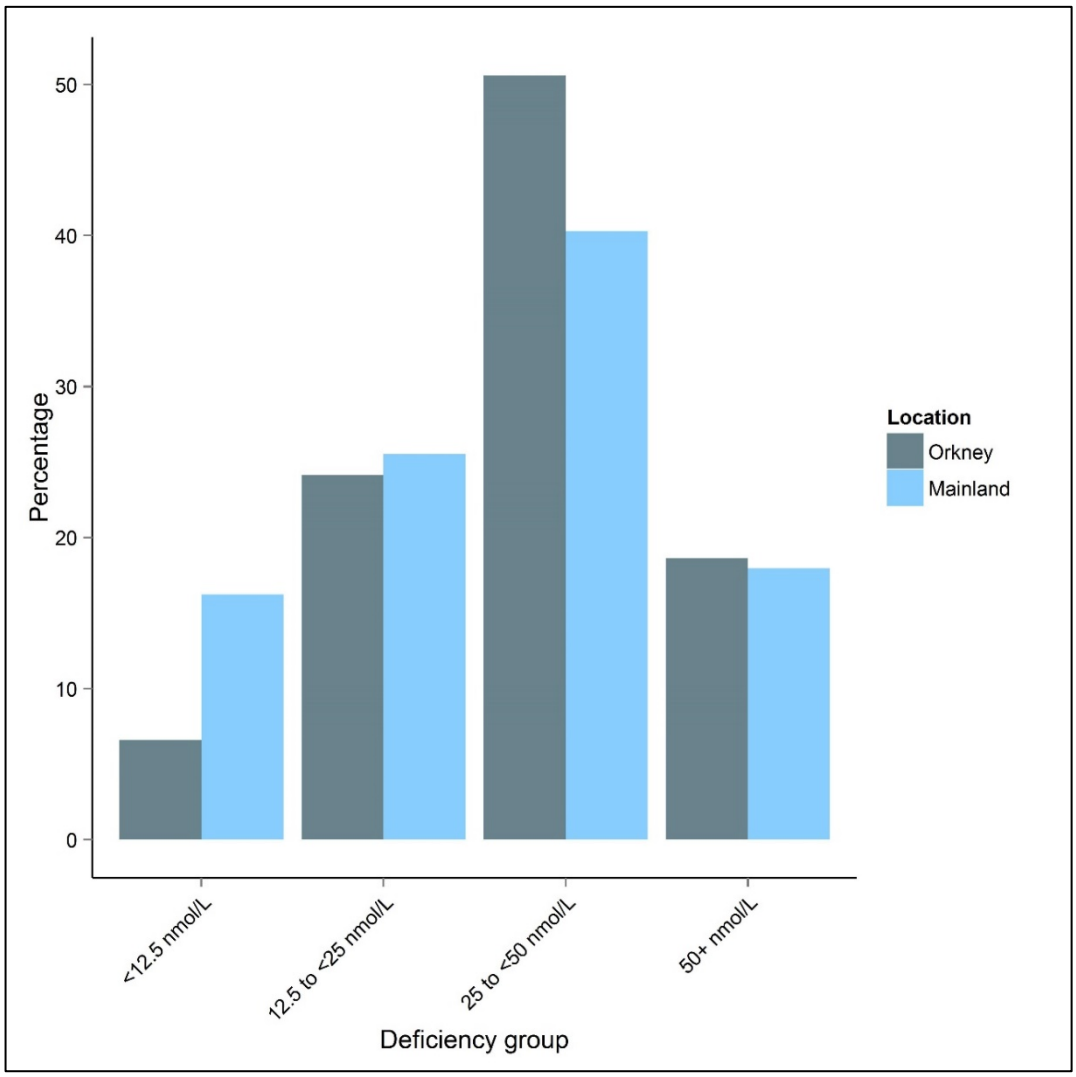


Figure 7.4 Comparison of percentage of people in May-adjusted vitamin D deficiency groups by location. The main differences occur in the severely deficient group (<12.5 nmol/L) which has significantly fewer people from the Orkney sample ($\chi^2(1)=64.2$, $p=1.10 \times 10^{-15}$), and the 'at risk' category (25-<50 nmol/L) which has significantly fewer people from the mainland Scottish sample ($\chi^2(1)=30.3$, $p=3.78 \times 10^{-8}$).




To explore correlates of vitamin D in Orkney we ran two multivariable regression analyses, using both imputed data and complete cases. Each model yielded similar results (Table 7.4). Variables significantly associated with higher 25(OH)D included

lower BMI, more foreign holidays, older age and increased PA. Associated with lower 25(OH)D was the “non-traditional” SES grouping.

Table 7.4 Results of linear regression for complete cases and imputed data using May-adjusted vitamin D as the outcome

| | **Multivariable models** | | | |
|---|---|---|---|---|
| | Model 1[a] (n = 628) | | Model 2[b] (n = 1949) | |
| **Predictors** | Est (95% CI) | p-value | Est (95% CI) | p-value |
| Intercept | 32.4 (18.5, 46.2) | $5.25 \times 10^{-6}$ | 30.3 (22.2, 38.3) | $2.2 \times 10^{-13}$ |
| Body mass index (kg/m$^2$) | -0.75 (-1.02, -0.47) | $1.95 \times 10^{-7}$ | -0.54 (-0.70, -0.38) | $7.5 \times 10^{-11}$ |
| Holidays outside the UK | | | | |
| < once a year | -0.93 (-4.38, 2.52) | 0.59 | 0.75 (-1.34, 2.85) | 0.48 |
| Once year | 5.03 (0.20, 9.86) | 0.041 | 6.47 (3.47, 9.47) | 0.000024 |
| > once a year | 18.7 (11.3, 26.2) | $1.04 \times 10^{-6}$ | 13.5 (9.07, 18.0) | $3.4 \times 10^{-9}$ |
| Age at venepuncture | 0.24 (0.11, 0.36) | 0.0003 | 0.14 (0.07, 0.22) | 0.00030 |
| Physical activity | 1.66 (0.58, 2.75) | 0.003 | 1.42 (0.65, 2.19) | 0.00032 |
| Socio-economic status 3 ("non-traditional") | -2.10 (-3.57, -0.64) | 0.005 | -1.74 (-2.71, -0.78) | 0.00043 |
| Summer minutes outside | 0.0046 (-0.00398, 0.013) | 0.29 | 0.006 (-0.00028, 0.012) | 0.062 |
| Socio-economic status 2 | 0.32 (-1.30, 1.94) | 0.70 | 0.69 (-0.34, 1.72) | 0.19 |
| Vitamin D intake (μg) | 0.201 (-0.18, 0.60) | 0.30 | 0.14 (-0.17, 0.46) | 0.37 |
| Working (not retired) | -2.07 (-6.61, 2.47) | 0.37 | -0.18 (-2.45, 2.09) | 0.88 |
| Sex (male) | 1.06 (-1.55, 3.67) | 0.43 | -0.12 (-1.77, 1.52) | 0.88 |
| Socio-economic status 1 | -0.013 (-1.85, 1.82) | 0.99 | -0.075 (-1.17, 1.02) | 0.89 |

[a] Model 1 constructed using complete cases in the original dataset, $R^2$=0.204; [b] Model 2 constructed using 68 datasets with missing data completed by imputation (100 cycles) , $R^2$ = 0.111 (People missing outcome data were excluded from the imputation model).
Socio-economic status was derived from principal components analysis.

The association between older age and higher vitamin D required further exploration; we began by comparing foreign holiday-takers and their non-holidaying counterparts. We found that people over 50 were significantly more likely to take foreign holidays at least once a year compared to people under 50 (Table 7.5, Figure 7.5) ($\chi^2(1)=6.4$, $p=0.0083$). We termed this the 'Saga' effect. Additionally, we found that foreign holidays had a stronger effect on people over 50 who had their blood drawn in the low vitamin D (weaker UVB) season (October to March) compared with people who had their blood drawn in the high vitamin D (stronger UVB) season (April to September) (Table 7.6).

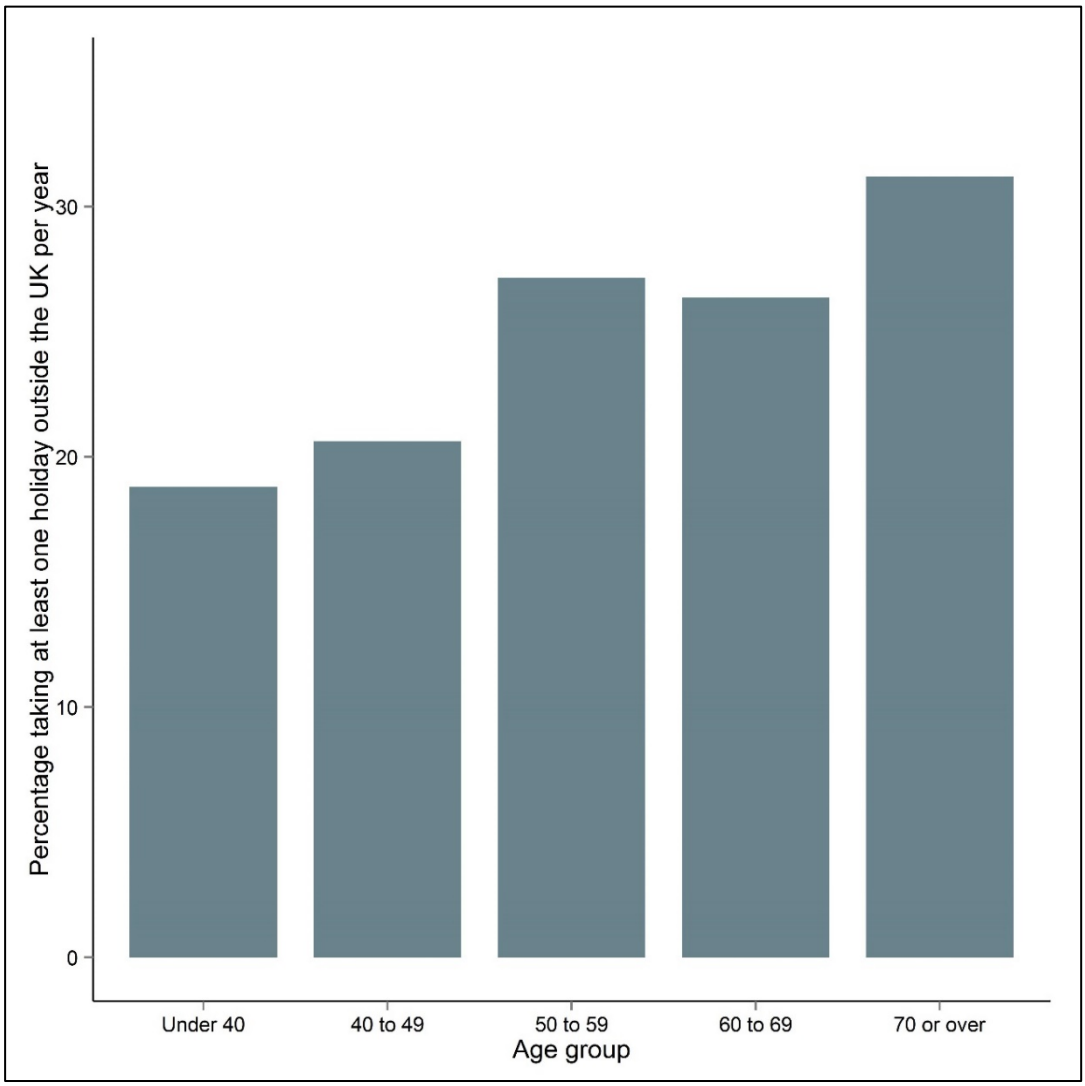
Table 7. 5 Comparison of people over 50 who holiday outside the UK at least once a year (n=281) and people over 50 who holiday outside the UK less than once a year or never (n=851). Unpaired t-tests applied to continuous data; chi-square tests applied to categorical data

| | **Over-50s, holiday No (%) or Mean (SD)** | **Over-50s, no holiday No (%) or Mean (SD)** | **t-test or $\chi^2$** | **p-value** |
|---|---|---|---|---|
| Socio-economic status 1 | 1.01 (0.775) | -0.299 (0.919) | -22.6 | $<2.2x10^{-16}$ |
| Job prestige score | 0.320 (1.08) | -0.192 (0.955) | -7.09 | $5.14x10^{-12}$ |
| Socio-economic status 3 ("non-traditional") | 0.182 (1.05) | -0.285 (0.915) | -6.50 | $2.13x10^{-10}$ |
| Supervisory role at work | | | | |
| Yes | 186 (66.4) | 377 (45.1) | | |
| No | 94 (33.6) | 459 (54.9) | 38.2 | $6.43x10^{-10}$ |
| Years in education | 15.9 (1.26) | 15.5 (1.14) | -5.39 | $1.13x10^{-6}$ |
| Body mass index ($kg/m^2$) | 27.6 (3.91) | 28.9 (5.03) | 4.41 | $1.19x10^{-5}$ |
| Highest qualification | | | | |
| O & standard grades, CSE | 33 (11.8) | 102 (12.1) | | |
| Highers, A levels | 114 (40.7) | 246 (29.3) | | |
| Certificates/diplomas | 113 (40.4) | 461 (54.8) | | |
| Bachelor/Master/PhD | 20 (7.14) | 32 (3.81) | 22.2 | $5.82x10^{-5}$ |
| Summer minutes | 252 (146) | 214 (136) | -3.38 | 0.000777 |
| Age | 62.9 (7.62) | 64.4 (8.63) | 2.55 | 0.0109 |
| Bodyfat % | 33.71 (7.51) | 34.9 (8.07) | 2.34 | 0.0197 |
| Socio-economic status 2 | 0.430 (0.758) | 0.316 (0.758) | -2.06 | 0.0400 |
| Vitamin D intake (µg) | 5.09 (3.60) | 4.78 (3.13) | -1.12 | 0.261 |
| Physical activity | 5.21 (1.20) | 5.16 (1.26) | -0.37 | 0.710 |

Table 7. 6 Mean May-adjusted vitamin D (nmol/L) according to season of venepuncture (high season (April-September, n=96) vs low season (October-March, n=185)) in people over 50 who take a holiday outside the UK at least once a year. Linear regression with May-adjusted vitamin D as the outcome

| | **Std Beta** | **Beta (95% CI)** | **Std error** | **p-value** |
|---|---|---|---|---|
| High season over-50s | 0.17 | 7.86 (3.57-12.15) | 2.18 | 0.000349 |
| Low season over-50s | 0.19 | 8.33 (5.23-11.43) | 1.57 | $1.75x10^{-7}$ |

Figure 7.5 Percentage of people per age group in ORCADES who holiday outside the UK at least once a year. People over 50 take significantly more holidays than those under 50




To explore under-40s further, we ran the same analyses comparing those who do and do not holiday outside the UK that were used for over-50s (S3 Table). The same results were significant, excepting body fat percentage, socio-economic status 2, age, and summer minutes spent outside.

We also identified a 'farmer effect' (Table 7.7). Participants employed in "traditional" agricultural occupations that kept them outdoors had significantly higher

mean vitamin D levels than participants in non-traditional professions that kept them indoors (farmers 36.9 (18.0), non-farmers 33.8 (11.8), $t(383)=2.46$, p=0.014). Further, farmers tended to be older.

Table 7.7 Comparison of farmers (n=265) and non-farmers (n=1649) on variables of interest in Orkney. Farmers are anyone who identified their primary profession as farmer. Unpaired t-tests applied to continuous data; chi-square tests applied to categorical data

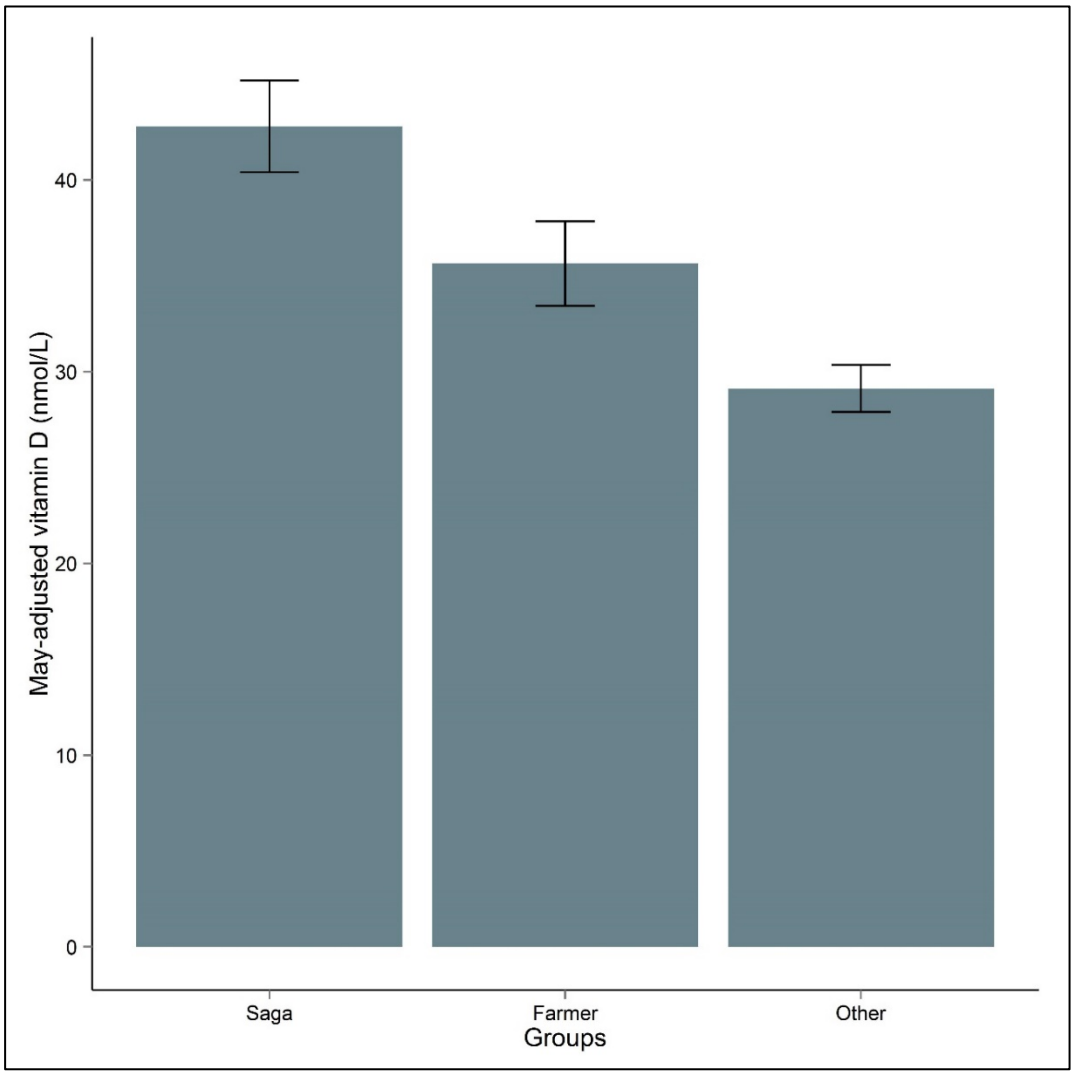
| | **Farmers No (%) or Mean (SD)** | **Non-farmers No (%) or Mean (SD)** | **t-test or $\chi^2$** | **p-value** |
|---|---|---|---|---|
| Age | 60.7 (11.4) | 52.4 (11.5) | -8.86 | $<2.2x10^{-16}$ |
| Socio-economic status 3 ("non-traditional") | -0.30 (0.58) | 0.10 (1.02) | 8.77 | $<2.2x10^{-16}$ |
| Years in education | 15.4 (1.01) | 16.1 (1.24) | 10.16 | $<2.2x10^{-16}$ |
| Socio-economic status 1 | -0.439 (1.00) | 0.0990 (0.973) | 7.86 | $5.39x10^{-14}$ |
| Physical activity | 5.87 (1.23) | 5.10 (1.21) | -7.60 | $8.89x10^{-13}$ |
| Highest qualification | | | | |
| O & standard grades, CSE | 24 (9.16) | 251 (15.3) | | |
| Highers, A levels | 84 (32.1) | 702 (42.8) | | |
| Certificates/diplomas | 153 (58.4) | 586 (35.8) | | |
| Bachelor/Master/PhD | 1 (0.382) | 100 (6.10) | 55.9 | $4.29x10^{-12}$ |
| Bodyfat % | 30.4 (8.65) | 33.1 (8.63) | 4.51 | $9.05x10^{-6}$ |
| Supervisory role at work | | | | |
| Yes | 109 (41.3) | 866 (53.2) | | |
| No | 155 (58.7) | 763 (46.8) | 12.8 | 0.000342 |
| Socio-economic status 2 | 0.281 (0.917) | 0.0672 (0.921) | -3.39 | 0.000771 |
| Body mass index ($kg/m^2$) | 28.4 (4.80) | 27.7 (4.96) | -2.12 | 0.035 |
| Vitamin D intake (μg) | 4.71 (2.93) | 4.40 (3.20) | -1.29 | 0.19 |
| Summer minutes | 236 (151) | 221 (141) | -1.21 | 0.225 |

* School examinations taken in the UK at ~16 years of age
** School examinations taken in the UK at ~18 years of age

To test for differences in mean vitamin D across the 'Saga' group, farmers, and non-farmers who are under 50 and do not take foreign holidays, we did a one-way ANOVA. This ANOVA was significant: Welch's $F(2, 481.99) = 54.49$, $p = 2.2 \times 10^{-16}$, and

we therefore concluded that vitamin D varies significantly across these groups with people over 50 who take foreign holidays having higher vitamin D than farmers, who had higher vitamin D than non-farmers and people under 50 who remain in the UK (Figure 7.6).

Figure 7.6 Mean May-adjusted vitamin D (nmol/L) in different groups in ORCADES. 95% confidence interval bars are given. "Saga" refers to people over 50 who take a holiday outside the UK at least once a year; "Farmer" to people who identified their primary profession as farming; "Other" is people under 50 who take a holiday outside the UK less than once a year, and are not farmers



### 7.2.4 Discussion

We aimed to compare vitamin D levels in Orkney and mainland Scotland, and to identify the determinants of Orkney vitamin D. Definitions of vitamin D deficiency are much discussed, however it has been proposed that circulating 25(OH)D above 50 nmol/L are sufficient (Ross et al., 2011; Holick et al., 2011). Vitamin D status in Scotland has been previously explored (Zgaga et al., 2011). Deficient and high risk individuals comprised 63.4% in the previous study; in our Orkney dataset deficient and high risk individuals comprised 65.3%. However, people with severe deficiency (<12.5 nmol/L) comprised 11.8% of the former study and only 5.0% of the latter. Therefore although perhaps initially surprising that mean vitamin D was higher in Orkney despite the higher latitude, the smaller percentage of people with severe deficiency in Orkney led to this elevation. In both datasets the majority are either deficient or at risk of deficiency which could have significant health implications (Holick, 2004a).

Ability to synthesise vitamin D decreases with age (MacLaughlin and Holick, 1985); it is well established that older age is associated with lower vitamin D and increased deficiency risk (Zgaga et al., 2011; Mithal et al., 2009). However, we found that lifestyle factors particular to Orkney contributed to better vitamin D in older compared to younger people.

Participants who took foreign holidays had higher vitamin D than those who did not take foreign holidays; furthermore, taking foreign holidays increased by age group. Less than 20% of under-40s in our Orkney sample took foreign holidays, whereas over 30% in the 70 and over group reported leaving the UK at least once a year. Weak sunshine within the UK leads to fewer opportunities for UV exposure and UVB-mediated vitamin D synthesis, and the effect of foreign holiday sun exposure has been previously associated with improved vitamin D in Scotland (Mavroeidi et al., 2013). Orkney mean vitamin D remained higher than mainland Scotland throughout

the year with the exception of two months: August and November. Although we were unable to explore this further, mainland Scotland's August elevation of vitamin D could be attributed to the effect of holidays, following one month after school holidays. In November, however, mainland Scotland's mean vitamin D levels were only minimally higher than Orkney. We found that mean vitamin D in people 50 and over taking foreign holidays was significantly higher than vitamin D levels in the rest of the sample. Foreign holidays contributed more to vitamin D levels on blood drawn in months of weaker UVB. We were unable to explore at what time of year people take holidays, however this finding suggests that foreign holidays become more important as a source of UV exposure and therefore vitamin D for Orkney residents in winter. As older people tend to have more freedom to travel outside peak season, they are able to derive most benefit by seeking sun in seasons of scarce sunshine in Orkney. People over 50 who take foreign holidays were found to differ from those who do not take holidays mainly in variables denoting financial security. They were also more likely to have lower BMI and body fat percentage suggesting possible healthier lifestyles than their non-holidaying counterparts.

We also examined under 40s, the age group in which MS is most likely to be diagnosed and pregnancies are most likely to occur, thereby potentially conferring risk to the unborn child. In this group, we found that the main differences in those who do or do not take holidays out of the UK are related to financial security. Only 75 of the 400 people who are under 40 reported leaving the UK for a holiday at least once a year, therefore, in the most at-risk group, inadequate UV exposure in Orkney is compounded by a low prevalence of foreign holidays.

The 'non-traditional' SES group derived from PCA, comprising job prestige score, education years and supervisory role at work, was associated with vitamin D.

These variables, reflecting "non-traditional" lifestyles of managerial, administrative, and professional occupations in contrast to traditional agricultural work, related to farmers and non-farmers. Farmers were found to be slightly less educated, possibly as a result of leaving school at the minimum leaving age about half a year to a year before the first set of examinations. Farmers were also less likely to describe themselves as having a supervisory role at work than non-farmers, and also had a slightly lower-than-average job prestige score. The inverse association between vitamin D and a higher score in this variable means that farmers, who scored lower, had better vitamin D. Farmers in our cohort were also more likely to be older than non-farmers, further contributing to our finding of vitamin D increasing with age.

Physical inactivity and obesity have previously been related to low vitamin D in a large American cohort (Brock et al., 2010); the association between lower BMI and higher 25(OH)D is also well established (Jorde et al., 2010). The mechanism for lower vitamin D in the presence of higher BMI is thought to be a result of increased deposition of vitamin D in body fat (Wortsman et al., 2000), making BMI a proxy for adiposity. However, BMI does not distinguish between body fat and fat free mass, and is not always a reliable indicator of adiposity in people with lower body fat but greater muscle mass (Wellens et al., 1996). The farmers in our cohort reflected this difficulty: they had lower body fat percentage but higher BMI than non-farmers. We found farmers were more active and leaner than non-farmers and it is perhaps therefore fair to assume they have higher than average muscle mass. Nevertheless, in the multivariable models BMI followed what is expected.

Farmers had mean vitamin D significantly higher than non-farmers, but significantly lower than people over 50 who take foreign holidays. Summer minutes outside was not significant in the multivariable analyses, however we performed a t-

test for farmers versus non farmers and time spent outside. Farmers, perhaps unsurprisingly, were found to spend significantly more time outside than non-farmers which enables maximisation of even the smallest window of vitamin-D strength sunshine. Although Zgaga et al. found higher vitamin D consumption led to slight improvements in plasma vitamin D (Zgaga et al., 2011), we found that diet was not associated with vitamin D in Orkney. However, difficulties involved in building a variable with the available data likely contributed to this finding.

Both studies were recruited on an 'opt in' basis which may result in the samples representing a healthier than average population; however a strength of this study was the large number of participants in each cohort. Particularly novel was the number of farmers in our Orkney cohort, enabling us to explore vitamin D in a select group within a rural population which is not often studied. All vitamin D samples from both cohorts were analysed in the same laboratory using the same procedures, helping maintain consistency and reliability of results. We had access to a variety of detailed measures to explore vitamin D in Orkney; however data on time spent outside and vitamin D intake were somewhat limited and these may thus be more strongly implicated in vitamin D than we were able to detect. Nevertheless, we found significant effects and reliable relationships for vitamin D with foreign holidays, BMI, physical activity, and age.

### 7.2.5 Conclusion

Mean vitamin D in Orkney was higher than mainland Scotland, driven largely by a lower percentage of individuals with severe deficiency in Orkney. Overall concentrations in both cohorts were low with most people either deficient or at risk of deficiency, suggesting that UV exposure for much of the year is low. Older Orkney residents were more likely to have better vitamin D than younger residents, largely resulting from the 'Saga' and 'Farmer' effects. Those most at risk of deficiency in Orkney were under 40, an age group traditionally considered at lower risk of deficiency, but at

increased risk of MS diagnosis. Within these main child-bearing years, a lack of UV exposure and vitamin D deficiency may result in significant autoimmune implications for offspring. The significant contribution of foreign holidays to Orkney vitamin D is consistent with the findings of previous UK studies; the importance of foreign holidays in providing adequate UV exposure to UK residents is underappreciated. We have found that younger ages are more at risk from inadequate UV exposure and vitamin D deficiency in Orkney, a county with a very high prevalence of MS. Further research exploring the relationship between vitamin D and quantitatively-measured exposure to UV radiation from sunshine and physical activity, as well as more detailed dietary information in Shetland, the most northerly UK county with an MS prevalence lower than Orkney, would help further elucidate the roles of UV exposure and vitamin D as MS risk factors in these islands.

## 7.3 Conclusion

As discussed, mean vitamin D in Orkney was higher than in mainland Scotland. It is possible, however, that this difference resulted from differences in the handling of the samples following blood draw and before analysis, and also in the laboratory procedures for analysis. The SOCCS study ran between 1999 and 2006. Blood in the SOCCS study was analysed for vitamin D in one batch in late 2008 or early 2009 (personal communication, Vaughan-Shaw, 20 November 2017), and the oldest samples would have therefore been stored for up to ten years. ORCADES ran between 2005 and 2011. Blood was analysed for vitamin D in one batch in 2011, and therefore the oldest samples would have been stored for up to six years (personal communication, Wilson, 15 November 2017). Both sets of samples were processed and frozen. The SOCCS samples were frozen at $-80^0$; the ORCADES samples were frozen at $-40^0$ and then $-80^0$ until analysis. Although there was a difference in both the length of time and temperatures at which samples were stored, studies have shown that vitamin D is a

resilient metabolite for long-term storage and is not adversely affected by differences in temperature, even when not frozen or refrigerated, over the short-term (Colak et al., 2013; Lissner et al., 1981; Hollis, 2008),

One further potential complication in comparing these samples from different studies is that they were analysed at different times. This could introduce some intra-laboratory variation, which could potentially lead to better detection of vitamin D levels in Orkney, therefore making it appear that mean Orkney vitamin D was higher. However, as shown in Table 7.3, there was a strong age effect whereby higher vitamin D levels were found in the younger population in the mainland, and in the older population in Orkney. This finding could be explained by the effect of holidays and outdoor working. Furthermore, when examining vitamin D by month, the mainland is higher than Orkney in August, which may also be explained by the effect of holidays following on one month after the end of school holidays (although data to test this were unavailable). It therefore seems unlikely that there was a systematic difference in measurement between the samples from the two studies as different age groups and different months show different patterns.

Finally, although the samples were analysed at different times, the ORCADES team specifically chose the same laboratory, the Scottish Trace Element and Micronutrient Diagnostic and Research Laboratory (STEMDRL), as the SOCCS study for analyses of blood vitamin D (personal communications, Vaughan-Shaw, 20 November 2017; Wilson, 15 November 2017). As well as using the same liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, the same laboratory would also be subject to the same quality control procedures, instrument maintenance and equipment calibration, which are important in ensuring as much consistency and therefore comparability between studies as possible (Black et al., 2015).

Methods for blood vitamin D measurement in STEMDRL changed in 2014, which was subsequent to all analyses for these studies (personal communication, Vaughan-Shaw, 20 November 2017). Therefore, whilst it should be noted that there are some differences in the handling of samples and different times of analysis that could lead to some differences observed between SOCCS and ORCADES datasets, care has been taken minimise such variation. Again, as noted above, vitamin D is a stable metabolite (Lissner et al., 1981; Hollis, 2008), and so it seems unlikely that these differences would have led to the observed results.

It is important to note that this study of vitamin D levels in Orkney has shown that vitamin D deficiency is particularly prominent in younger people in a population which has a high prevalence of MS. From the literature, and from our study of the heritability of MS in Orkney, there is clearly a requirement for a risk factor which increases the risk of MS in genetically susceptible individuals. Vitamin D deficiency in Orkney is a strong candidate for one such environmental risk factor.

Additionally, UV exposure itself may be important in mitigating MS risk. To further understand how UV exposure may be affecting the populations in such high-latitude islands Chapter 8 explores individually-measured UV exposure in Shetland, north of Orkney.

# CHAPTER 8: THE VIKING UV STUDY

## 8.1 Introduction

Following the Orkney vitamin D study, we explored how individual and lifestyle factors contribute to UVB exposure in Shetland. In this chapter I describe the methods and present the results of the Viking UV study. I discuss our findings in the context of the wider literature and their implications for the population of Shetland. However, I firstly describe UVB's place in the spectrum of radiation from the sun, and discuss environmental and personal factors known to affect our exposure to UV. I then define how vitamin D-strength UV exposure is measured, and briefly summarise the importance of UV exposure to human health.

### 8.1.1 Sunlight and UV radiation

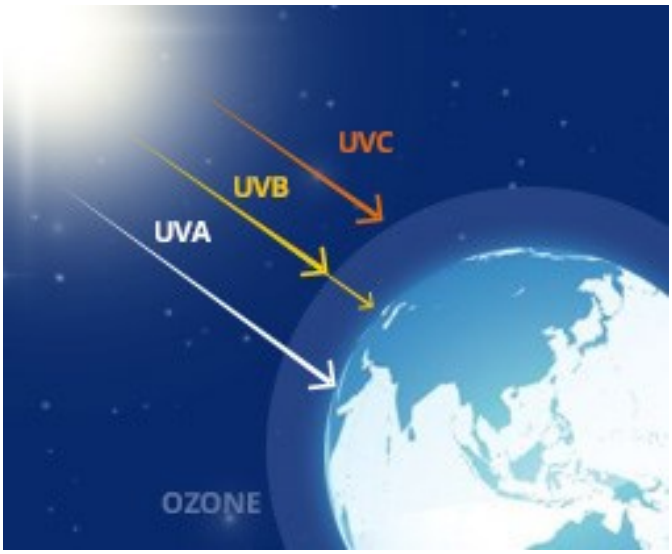
Sunlight comprises a range of electromagnetic radiation of various wavelengths. The wavelengths are used to categorise sunlight into three groups: infrared radiation, visible light, and UV radiation. However, because these groups are all part of a larger continuum (the electromagnetic spectrum), the divisions between bands are not exclusive, and some overlapping of wavelength occurs. The UV spectrum spans wavelengths from 200-400nm, shorter than visible light (400-700nm), and infrared radiation (700nm to 1mm). UV itself is furthermore divided into three categories dependent on wavelength: UVA (320-400nm), UVB (280-320nm), and UVC (100-280nm) (Orteu et al., 2001).

UVC is fully absorbed by ozone and does not reach the Earth's surface (Figure 8.1). UVB is filtered by ozone; however, UVB rays make up between 5 and 10% of all UV radiation at the Earth's surface. In humans, UVB is absorbed by epidermal skin and penetrates through to the papillary dermis. It causes erythema and sunburn. Over the long-term, UVB exposure can cause non-melanoma and contributes to risk of

melanoma. However, UVB is also the principal source of vitamin D in humans (Orteu et al., 2001; Holick and Jenkins, 2009).

UVA comprises 90-95% of all UV radiation at the Earth's surface. It penetrates ozone and infiltrates the skin more deeply than UVB. Unlike UVB, UVA is also able to penetrate water and glass. Excessive UVA exposure increases risk of photoaging and melanoma (Narayanan et al., 2010).

Figure 8.1 UV rays and the earth's surface

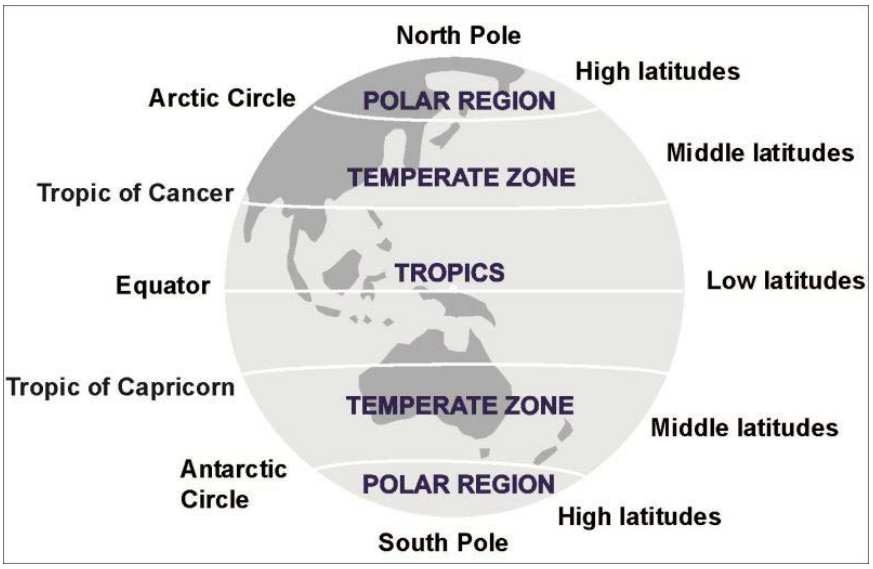



*(creativecommons.org/licenses/by-nc-sa/2.5/au/legalcode, Energizer Australia (2017))*

### 8.1.2 What affects our exposure to UVB radiation?

Two groups of factors, which I will term environmental and personal, affect our exposure to UV radiation. Environmental factors affect the amount of UVB available on the Earth's surface. These include the solar zenith angle, ozone, weather conditions, surface reflectivity, latitude, season, and time of day. The definitions of high and low latitude are shown in Figure 8.2. Personal factors include sunscreen use, clothing cover, and sun-seeking or sun-avoidance behaviours. Vitamin D synthesis is also dependent upon these factors, and additionally is affected by skin colour and age (Webb, 2006).

Figure 8.2 Latitude regions. Polar regions are high latitude, temperate zones are mid-latitude, and the tropics stretching across the equator are low latitude




(Bureau of Meteorology Training Centre, 2017)

#### *8.1.2.1 Environmental factors*

The solar zenith angle (SZA) is the angle between a hypothetical line that ascends vertically from any location on earth, and the position of the sun in the sky. The SZA is affected by the time of day, time of year, and latitude. Small SZA are associated with summer months, midday hours, and low latitude areas, whilst large SZA are characteristic of winter months, early morning and late evening hours, and high latitude regions (Webb, 2006). Small SZA are favourable for UVB infiltration and subsequent vitamin D production. This is because when the sun is high in the sky, there is less distance for the rays to travel through the atmosphere, and the resultant energy is concentrated on a relatively small area. Large SZA are less beneficial as the radiation has a longer path through the atmosphere; there is more chance that the radiation will be attenuated, and additionally, at the Earth's surface, the energy is spread over a large area (Webb, 2006). At high latitudes, winter sunshine is insufficient to initiate vitamin D synthesis (Holick, 2004a; Webb and Engelsen, 2006).

The SZA therefore has an important role in the amount of potential UV that can reach the Earth. However, several other, less predictable, environmental factors can inhibit UVB infiltration. As mentioned previously, ozone absorbs UVB. Ozone is found principally in the stratosphere and follows a global pattern with the least ozone occurring around the tropics and the most at the edge of the polar regions. Between these two extremes, the amount of ozone increases with latitude. Ozone is also subject to seasonality. At mid-latitudes, ozone is at its minimum in the summer and winter months and at its maximum in spring and autumn (Webb, 2006). Lower levels of ozone result in greater potential for vitamin D synthesis by UVB (Kimlin, 2008).

As well as ozone, UV radiation can be scattered by other atmospheric particles, including oxygen, nitrogen, aerosols, and water vapour. Low-level air pollution also reduces UV levels, particularly in urban environments (Kimlin, 2008). Areas of high altitude also usually have greater UV infiltration than low altitude areas because the distance for the rays to travel is smaller and there is less chance of attenuation by these atmospheric particles.

Finally, cloud cover can have a significant impact on UV infiltration. The amount of UV that reaches the Earth's surface is variable depending upon the density of cloud cover, and the size, shape, and composition of the clouds. Light clouds that do not cover the sun are unlikely to impact the UV reaching the earth. However, overcast skies significantly reduce UV (Kimlin, 2008; Webb, 2006). Conversely, in certain circumstances, clouds can briefly increase the amount of available UV (Kimlin, 2008). In areas of high surface albedo or reflectivity, for example newly-laid snow, some radiation is reflected from the surface. This reflected UV may then be back-scattered by the atmosphere, particularly if a light layer of cloud is present. The back-scattering

returns the UV to the Earth's surface thereby increasing ambient UV (Kimlin, 2008; Webb, 2006).

Environmental factors clearly impact how much UV reaches the Earth's surface. However, personal reasons affect the amount of UV to which we are exposed.

#### *8.1.2.2 Personal factors*

Personal factors comprise behaviours which affect the amount of UV that reaches our skin. Clothing cover and sunscreen both provide barriers which block UVB, and thereby prevent cutaneous vitamin D synthesis. Holick (2004b) estimated that when following guidelines for proper use, SPF15 reduces vitamin D synthesis by over 98%, and SPF8 by up to 95%. Additionally, the amount of time spent outdoors, the period during the day spent outdoors, and the amount of skin exposed to the sun, all affect UVB exposure. As mentioned above, summer midday hours are most efficient for UV's infiltration to Earth, and therefore exposing skin to sunlight during these times maximises the amount of UVB that it is possible to receive. However, it is important to note that prolonged exposure is not beneficial to vitamin D levels, and is likely to be harmful (Webb, 2006; Byrne, 2014). Instead, short, regular periods of exposure are sufficient to increase vitamin D levels. People who avoid the sun, or who cover their skin with sunscreen or clothing, are at more risk of vitamin D deficiency, and will not gain other health benefits from sunlight.

Age and skin type are two other important factors which can result in low vitamin D levels regardless of UVB exposure. As mentioned in Chapter 7, the ability to synthesise vitamin D decreases with age, as the amount of 7-dehydrocholesterol (7-DHC) in the skin is reduced (MacLaughlin and Holick, 1985). Age therefore comes with an increased risk of vitamin D deficiency (Zgaga et al., 2011; Mithal et al., 2009).

Skin type also affects the amount of UVB that can initiate vitamin D synthesis. Darker skins contain more melanin which absorbs UV radiation. This absorption protects skin from sunburn, however it also means that UV is effectively prevented from entering other skin cells, thereby reducing the amount of 7-DHC that can be converted into previtamin $D_3$. People with dark skins who live in high-latitude regions are therefore at particular risk of vitamin D deficiency (Webb, 2006).

### 8.1.3 Measurement of UVB

One sign that skin has been exposed to adequate sunlight for vitamin D synthesis is erythema, defined as a just-perceptible reddening (but not burning) of the skin (Webb, 2006). However, simply measuring UV exposure is not informative of the erythemal action of the exposure (CIE Standard, 1998). UV radiation that elicits erythema can be measured using the minimal erythema dose (MED). The MED reflects individuals' sensitivity to UV, dependent on factors including skin pigmentation, previous UV exposure, and anatomical site. The MED is therefore different for each individual. Furthermore, there are observational difficulties including how to define the erythema (CIE Standard, 1998). However, Lo et al. (1986) found that when the MED had been met in people of different skin types – in this study Indian and Pakistani immigrants to the UK compared to UK residents of European heritage – the amount of vitamin D produced was equivalent.

Despite its uses, the subjective nature and lack of standardisation of the MED was problematic when trying to use it as a measure of UV radiation (Diffey, 2002b). The standard erythema dose (SED) was therefore proposed and is widely used as an erythemally-weighted UV radiation measurement. One SED is equivalent to an erythemal effective exposure of $100 J^{m-2}$ (CIE Standard, 1998). To give an indication of the practical meaning of this measure, Diffey (2002b) provides the following examples: a) daily ambient exposure on a summer's day in Europe with a cloudless sky would be

around 30-40 SED; b) 4 SED would elicit moderate erythema on white, previously unexposed skin, but would not produce erythema on skin that had been previously exposed.

### 8.1.4 UVB radiation and health

The effectiveness of using sunlight to treat conditions such as tuberculosis (Gardiner, 1915; Holmboe, 1916), psoriasis (Alderson, 1923), and rickets (Chick et al., 1922), were well-recognised before the biological actions of UV exposure were understood. Since then, our understanding of how the different wavelengths of UV confer different biological effects, and of the importance of adequate UVB exposure to human health, has grown.

Underexposure to UVB has been cited as a risk factor for many diseases, including but not limited to, several types of cancers (Grant, 2002; Boscoe and Schymura, 2006), cardiovascular disease (Holick, 2004a; Holick, 2002), bone health including rickets and osteoporosis (Holick, 2004a), and autoimmune diseases including rheumatoid arthritis, type 1 diabetes mellitus, and MS (Ponsonby et al., 2002). Underexposure has also been associated with increased mortality from cancer (Grant, 2002).

The concomitant reduction in vitamin D levels with lower levels of UVB exposure is considered a risk factor for many of these conditions. A 2009 study estimated that maintaining sufficient vitamin D levels (40 ng/mL) would reduce the economic burden of disease by €187,000 per year in Western Europe (Grant et al., 2009).

However, as discussed in Chapters 6 and 7, UVB exposure also has immunosuppressive properties. UV induces the innate immune system, thereby protecting from events such as microbial infections. It also suppresses the adaptive

immune system, thus reducing autoimmune and allergic responses (Schwarz, 2010). Several authors have linked this reduced autoimmune response to a reduced risk of MS (Wang et al., 2013; Lucas et al., 2011; Tsunoda et al., 2005). However, immunosuppression from UV, particularly in the UVB range, has been linked to adverse health outcomes such as risk of melanoma in individuals who show particular susceptibility to UV (Yoshikawa et al., 1990). To maintain optimal health, adequate but judicious UVB exposure seems to be vital, for both its immunosuppressive properties and for its role in vitamin D synthesis.

Our study of vitamin D in Orkney found that foreign holidays are an important contributor to the population's vitamin D levels. However, farming was also associated with higher vitamin D levels. Farming involves greater-than-average time spent outside, meaning that the strength of Orkney UV can be sufficient to initiate vitamin D synthesis. To understand how local lifestyles may affect individual exposure to UV and, potentially, vitamin D levels in the highest-latitude county of the UK, we ran the Viking UV Study in Shetland.

### 8.1.5 Aims

Our aim was to investigate UV exposure in Shetland. We had two objectives to achieve this aim.

1) Identify correlations between UV exposure and a range of personal and lifestyle factors.
2) Determine how these factors may each contribute to individual UV exposure in Shetland.

## 8.2 Methods

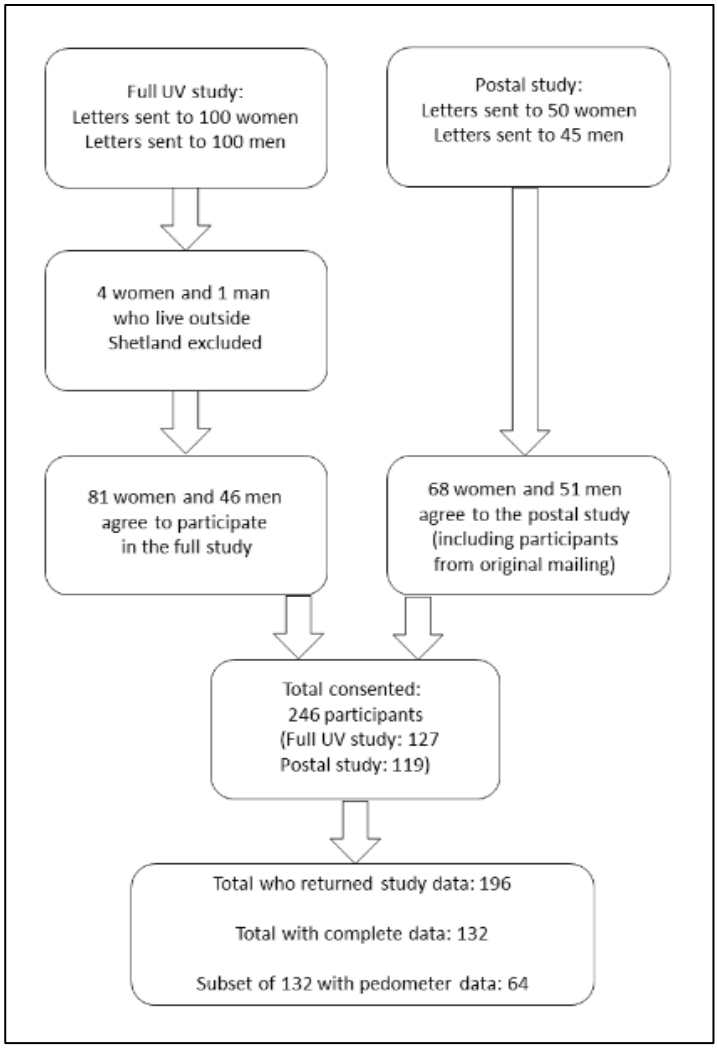
### 8.2.1 Study Population

All UV study participants had previously consented to take part in the Viking Health Study Shetland (VIKING). Like ORCADES, VIKING is a cross-sectional genetic

epidemiology study involving around 2000 Shetlanders, and is concerned with identifying genetic factors that influence complex disease. Data were collected at a measurement clinic where biometric measurements were recorded and blood was drawn. Additional data included cognitive function tests and a comprehensive health survey.

There were two phases of recruitment for the UV study. Firstly, we identified a subset of 200 VIKING participants who had not yet attended their clinic appointment, and approached them by mail. One hundred and twenty-six people registered interest. Available participants were booked into the VIKING clinic from 12th June 2014 to 2nd September 2014. Participants who were unable to attend a clinic during these times agreed to receive a study pack and instructions by post and to mail back the data. To increase our sample size, we identified in the same manner and approached an additional 95 VIKING participants by mail and asked them to participate in the postal version of the study. Study recruitment is illustrated in Figure 8.3.

Figure 8.3 Flow chart illustrating the recruitment of the Viking UV study




Inclusion criteria for the UV study comprised VIKING participants aged between 18 and 70. Fourteen participants aged between 71 and 78 were also contacted and agreed to participate; ten returned completed study data before the oversight was noticed, and we therefore decided to include these data in the analyses. Individuals were also excluded if they had taken a holiday outside of Shetland, or had used a sunbed within two months prior to the data collection. This time limit was based on the vitamin D half-life (Mawer et al., 1971).

### 8.2.2 Data collection

The UV study ran between weeks beginning 16th June 2014 and 3rd September 2014. Each participant contributed data collected over a period of one week. The start date of the data collection week varied depending upon when participants visited the clinic or when they received their postal study pack. Data for the UV study consisted of measures of UV exposure and physical activity alongside personal characteristics such as skin type. The UV study data were then combined with VIKING data. Details of all data are below.

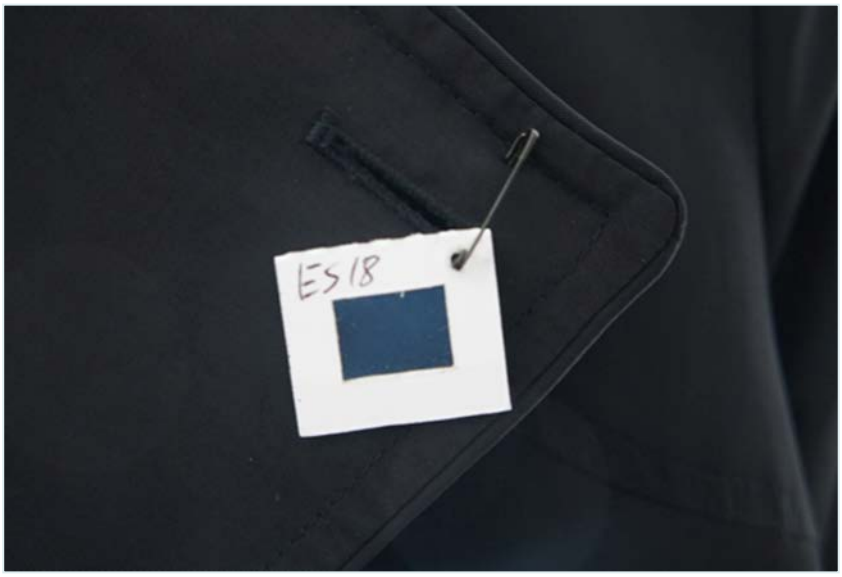
#### *8.2.2.1 UV measurement*

Each participant was either sent, or, if they attended a clinic, given, a polysulphone badge (Figure 8.4). Polysulphone badges consist of a square of cardboard with an aperture of polysulphone film. On exposure to UVB radiation, the UV absorbance of the film increases. The UV absorbance is measured before and after exposure at 330nm in a UV spectrophotometer to give a measure of the UV dose, which is calculated as a continuous variable in SEDs. We ordered the badges from Dr Richard Kift of Manchester University and returned them to him for measurement following exposure.

We supplied one badge per participant. Participants were instructed to wear their badge on their right or left lapel every day for one week, ensuring that it was not covered by clothing. We chose the lapel site because it is an area where the badge can be easily attached and be unobstructed by clothing cover. Other studies have used the shoulder/chest site for polysulphone dosimetry (Kimlin et al., 1998a; Kimlin et al., 1998b; Cahoon et al., 2013; Diffey et al., 1996). Additionally, as there is a strong correlation between radiation received at the right and left side of the upper chest, we considered that we need not specify exact badge placement (Waterston et al., 2004). Before use, each badge was kept in an individual black gabardine pocket concealed

inside a small brown envelope. This was to ensure that no UV light entered the badge before commencement of the study. Following the end of the study, participants were asked to return the badges to the black pocket, place and seal them inside the brown envelope, and either hand them into the clinic or send them back to us in a prepaid envelope that we supplied.

Figure 8.4 Polysulphone badge




We asked all participants to keep a sun exposure diary for the duration of their study week (Appendix E). We asked how much time each day had been spent outside and during which time periods, beginning with 'before 8.30am', and every hour thereafter until the final category of 'after 5.30pm'. We also asked how much skin was exposed during these outdoor periods (face, hands, head, arms, legs, feet, and torso). We asked whether participants had been wearing sunscreen which we recorded as a dichotomous (yes/no) variable. Other questions included whether participants considered their activity levels to have been 'normal' for the day of week and time of year. This was to determine whether participants were changing their behaviour as a result of participating in the study.

We calculated a variable from the sun exposure diary for 'time spent outside', which we weighted by the time periods during the day in which participants were outside. We refer to this variable as 'skin exposed (weighted)'. To calculate this variable, we used a score developed by Diffey (2002a), where we assigned a percentage of ambient daily UV per person per hour (Table 8.1).

Table 8.1 Daily UV percentage by hour, from Diffey et al. (1990)

| Time | Daily UV (%) |
|---|---|
| Before 8.30 | 6 |
| 8.30am – 9.30am | 8 |
| 9.30am – 10.30am | 12 |
| 10.30am – 11.30am | 15 |
| 11.30am – 12.30pm | 17 |
| 12.30pm – 1.30pm | 15 |
| 1.30pm – 2.30pm | 12 |
| 2.30pm – 3.30pm | 8 |
| 3.30pm – 4.30pm | 4 |
| 4.30pm – 5.30pm | 2 |
| After 5.30pm | 1 |

For example, if someone was outside for five minutes between 10.30 and 11.00, and again for thirty minutes between 1.30 and 2.30, they scored ((5/60) × 15) + ((30/60) × 12) = 7.25. We repeated this process for each day of the week and summed the daily totals to give a final score for each participant's study week.

Finally, we obtained satellite-measured ambient vitamin D-strength UV from the Tropospheric Emissions Monitoring Internet Service (TEMIS). These daily ambient vitamin D-strength UV measurements take into account cloud cover, and we obtained data for the complete study period from June to September 2014. We centred the measurements over Lerwick, the most populous town in Shetland, at coordinates 60.15

N and 1.14 W. From these data, we calculated the maximum amount of vitamin D-strength ambient UV that was available during each participant's study week.

#### *8.2.2.2 Lifestyle factors*

VIKING participants attended measurement clinics where height and weight were recorded, and body fat percentage was measured from a DEXA scan. We calculated BMI from the height and weight measurements. Each VIKING participant also completed medical and lifestyle questionnaires. Four of the usual 10 questions from the Fitzpatrick skin type scale were asked in the VIKING questionnaire. To derive a reliable variable for skin type we asked the remaining six Fitzpatrick questions as part of the UV study Sun Exposure Questionnaire (Appendix E). We collated data concerning the Fitzpatrick skin type questions from the separate sources, and categorised individuals into six groups according to their score. Those scoring 0-6 have skin type 1 (always burns); 7-13 skin type 2 (burns easily); 14-20 skin type 3 (burns moderately); 21-34 skin type 5 (rarely burns) and over 35 skin type 6 (never burns).

Physical activity data were recorded in VIKING. Each VIKING participant completed the long version of the self-administered International Physical Activity Questionnaire (IPAQ). The IPAQ is an internationally-validated questionnaire developed for use with adults between the ages of 15 and 69 (Craig et al., 2003). It comprises questions about activity undertaken during the previous seven days. Activity is divided into four domains: work, active transportation, domestic and garden, and leisure time. It further separates these domains into walking, moderate activity, and vigorous activity. Domain-specific and activity-specific scores can be calculated. For domain-specific scores, the separate totals for walking, moderate activity, and vigorous activity within each domain are summed. For activity-specific scores, the specific type of activity across all domains is summed. Activity scores can also be calculated as MET-minutes (metabolic equivalent of task). One MET is equivalent to the amount of energy

used by a seated individual at rest. Different types of activity can be weighted by their energy requirements to give the MET. The MET can then be multiplied by the number of minutes that the activity was performed, giving MET-minutes (IPAQ, 2013). We calculated all IPAQ activity scores in accordance to the IPAQ scoring protocol (IPAQ, 2013). We calculated activity as both a total of domain-specific activity, and activity in MET-minutes per week.

We collected additional activity data in the UV study from a limited number of pedometers, which counted the total steps taken. Participants of the UV study who were attending the measurement clinic for VIKING were given pedometers on a first-come, first-served basis. The pedometers were reset before distribution, and were sealed with masking tape. This was in an effort to reduce participants' knowledge of their step-counts and thereby reduce the possibility of score inflation. Each participant was given an information sheet with pictures demonstrating how the pedometer should be worn: horizontally on a waistband, halfway between belly button and hip. On their return to the clinic, participants handed the pedometer to the receptionist who recorded the step count for the previous seven days. We used the total of the first six days as the activity total as the final day was incomplete and depended upon the time at which the participant returned for their clinic appointment.

We used information from the VIKING questionnaire to construct indices for socio-economic status in the same manner as for ORCADES. Using PCA, we derived two variables from 9 questionnaire items, and the occupation question to which we assigned an occupational prestige score (Nakao and Treas, 1990) (Table 8.2). Years in education, highest qualification, job prestige score, and supervisory role at work loaded significantly onto the first component; housing tenure, council tax band, number of vehicles, and holidays within and outside the UK loaded significantly onto the second

component. The first component is therefore capturing education and professional roles while the second is concerned with financial security.

Table 8.2 Principal components analysis

| | PC1 | PC2 |
|---|---|---|
| Years in education | **0.79** | -0.10 |
| Highest qualification | **0.76** | -0.01 |
| Job prestige score | **0.65** | 0.08 |
| Housing tenure | -0.20 | **0.54** |
| Council tax band | 0.13 | **0.55** |
| Number of vehicles owned | -0.07 | **0.34** |
| Holidays in the UK | 0.21 | **0.56** |
| Holidays outside the UK | 0.21 | **0.62** |
| Supervisory role at work | **0.33** | 0.19 |
| Eigenvalue | 1.98 | 1.40 |
| % Explained | 20.9 | 16.3 |
| % Cumulative | 20.9 | 37.2 |

#### *8.2.2.3 Sample size*

Sample size was limited by three factors. These were 1) how many people could be seen at the VIKING measurement clinic; 2) the amount of time that VIKING had left to run, and 3) how long there would be sufficient UVB in Shetland. With these fixed parameters, we needed to work out what an acceptable level of accuracy would be. We therefore aimed to recruit 180 participants into the UV study. We considered this to be an achievable and realistic target which would enable us to detect correlations of 0.2 with a 95% confidence interval of 0.05 to 0.34.

### 8.2.4 Statistical analyses

#### *8.2.4.1 Relationship between individual UV and other variables*

To explore the data and examine the relationship between individual UV exposure (SED) and other variables we conducted bivariate Pearson correlations for continuous variables, t-tests for binary variables, and an ANOVA for the categorical variable. All analyses conducted herein are exploratory; as such, we have not adjusted

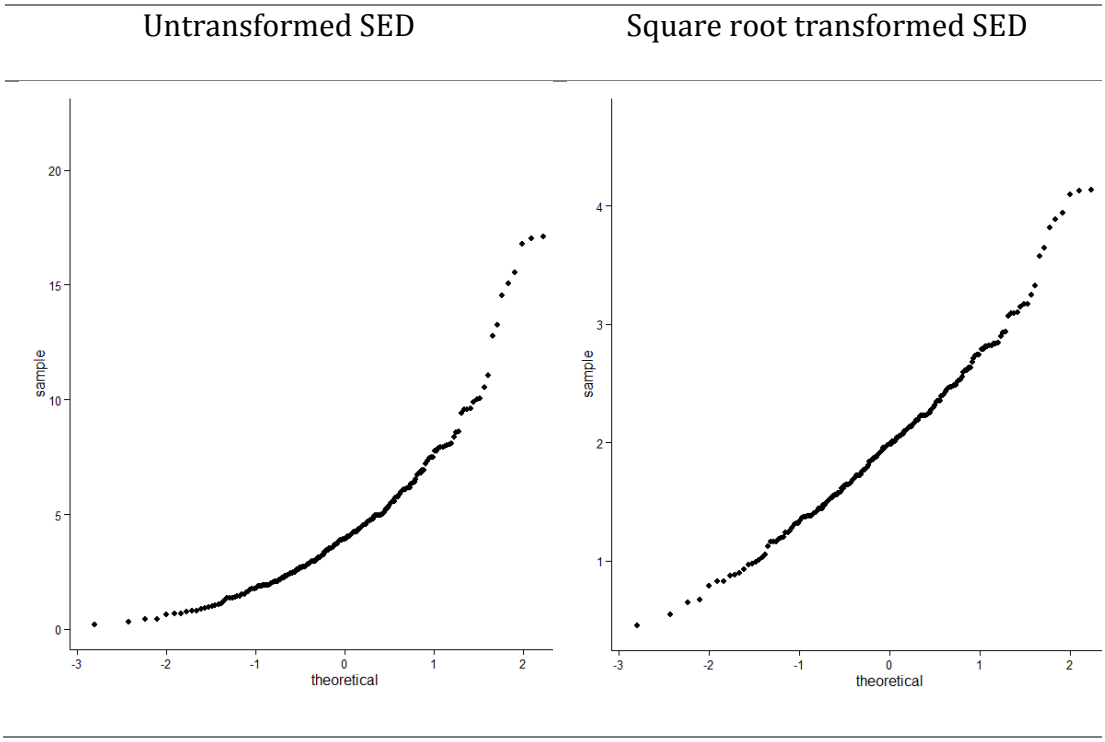
for multiple testing. This is because, in exploratory analyses, the number of tests have not been specified *a priori* and the precise number of familywise tests are undetermined. Furthermore, only a subset of tests are documented. This means that the nominal familywise error rate cannot be calculated with certainty. Attempting to adjust for multiple tests can then lead to the rejection of true associations (Rothman, 1990a; Bender and Lange, 2001; Rubin, 2017).

The variables that we tested comprised personal characteristics, including age, sex, skin type, skin exposed (weighted), indoor or outdoor occupation, working or retired, the two SES variables from PCA, and ambient UV. We also tested physical activity variables, including pedometer step count, and the totals for the IPAQ work, domestic, transportation, leisure, and walking domains, and the totals of moderate and vigorous MET minutes per week and the total IPAQ score. Finally, we tested weight and body fat variables including weight, waist and hip measurements, body fat percentage, and BMI.

#### *8.2.4.2 Bivariable and multivariable models*

Before conducting the bivariable and multivariable analyses we applied a square root transformation to SED to reduce the positive skew (Figure 8.5).

Figure 8.5 Square root transformation of UV dose




For determinants of UV exposure, we ran a series of bivariable models of SED against environmental and demographic variables of interest. Significant variables were put into multivariable models. These significant variables comprised sex, body fat percentage, indoors or outdoors occupation, IPAQ total score, total steps, Fitzpatrick skin type, skin exposed (weighted), TEMIS ambient UV, and SES1. We also included age. We ran two multivariable models. The first comprised the full sample size and used the IPAQ total score variable for physical activity. The second model comprised the subset of participants who had been given a pedometer, and, as well as including the IPAQ total score for physical activity in the model, we included total steps taken. We transformed all continuous variables into z-scores so that their mean was 0 and standard deviation equal to 1. This was to simplify interpretation of the results and to see how variables compare in their contribution to UV exposure.

We assessed homoscedasticity by inspection of a QQ plot, and distribution of residuals using a histogram. We checked independence using the Durbin Watson statistic, multicollinearity and outliers using the variance inflation factor (VIF) statistic and Cook's distance, respectively. All analyses were conducted using R software version 3.2.0 (R Core Team, 2015).

### 8.2.3 Ethical approval

Ethical approval was obtained for VIKING, and we applied for and were granted ethical approval for an amendment to the VIKING protocol from NHS Lothian. We completed a level 1 self-audit form in compliance with the Edinburgh University Research Ethics committee through which we identified no complex ethical issues requiring further scrutiny. All our participants gave their informed consent prior to the VIKING and UV study commencement.

## 8.3 Results

A total of 246 people consented to participate in the UV study, and were sent or given study packs consisting of a questionnaire and polysulphone badge. Of the 246 people who agreed to participate, 196 (80% of the total) returned data, and, of these people, 132 (54% of the total) completed the questionnaire and had worn their badge as instructed. Of these 132 participants, 64 (48%) had worn a pedometer. Characteristics of all 196 participants who returned study data are presented in Table 8.3.

Table 8.3 Participant characteristics excluding late or unreturned study packs (n=196)

| Characteristic | No or mean | SD | % or range |
|---|---|---|---|
| Age at time of study | 52.5 | 13.0 | 19.8 -78.7 |
| Under 30 | 13 | - | 6.6% |
| 30 to under 40 | 24 | - | 12.2% |
| 40 to under 50 | 38 | - | 19.4% |
| 50 to under 60 | 64 | - | 32.7% |
| 60 to under 70 | 40 | - | 20.4% |
| 70 and over | 16 | - | 8.2% |
| Sex | | | |
| Female | 122 | - | 62.2% |
| Male | 74 | - | 37.8% |
| Erythemal dose (SED) | 4.7 | 3.7 | 0.2 – 22.0 |
| Body fat | 31.8 | 8.3 | 10.5 – 50.5 |
| BMI | 27.4 | 4.6 | 18.7 – 46.3 |
| Occupation | | | |
| Indoor | 174 | - | 88.8% |
| Outdoor | 22 | - | 11.2% |
| Working/retired | | | |
| Working | 161 | - | 82.1% |
| Retired | 35 | - | 17.9% |
| Physical activity | 7665.0 | 6115.5 | 297 – 29178 |
| Skin type | | | |
| Skin type 1 | 0 | - | 0% |
| Skin type 2 | 11 | - | 5.6% |
| Skin type 3 | 47 | - | 24.0% |
| Skin type 4 | 101 | - | 51.5% |
| Skin type 5 | 30 | - | 15.3% |
| Skin type 6 | 0 | - | 0% |
| Skin exposed (weighted) | 34.0 | 29.5 | 1.3 – 185.1 |
| Total steps | 48,800 | 18.975 | 17,660 – 109,458 |

Table 8.4 compares people who did and did not return study data. For most variables, there is no significant difference between returners and non-returners. However, people who did not return study data had a significantly higher mean score

for the first PCA socioeconomic status variable. This corresponded to people with a higher socioeconomic status.

Table 8.4 Comparison of people who returned their study data (n=196) and people who did not return their study data (n=50). Unpaired t-tests applied to continuous data; chi-square tests applied to categorical data

| | **Returners** **No (%) or Mean (SD)** | **Non-returners** **No (%) or Mean (SD)** | **t-test or $\chi^2$** | **p-value** |
|---|---|---|---|---|
| Age | 52.5 (12.9) | 46.9 (21.7) | -0.6 | 0.5 |
| Sex | | | | |
| Female | 122 (62) | 27 (54) | 1.1 | 0.3 |
| Male | 74 (38) | 23 (46) | | |
| % Body fat | 31.8 (8.3) | 33.2 (9.1) | 1.0 | 0.3 |
| Occupation | | | | |
| Indoor | 174 (89) | 46 (92) | 0.4 | 0.5 |
| Outdoor | 22 (11) | 4 (8) | | |
| Skin type group | | | | |
| 2 | 11 (5.8) | 0 (0) | 1.5 | 0.7 |
| 3 | 47 (24.9) | 5 (27.8) | | |
| 4 | 101 (53.4) | 11 (61.1) | | |
| 5 | 30 (15.9) | 2 (11.1) | | |
| IPAQ total score | 7664.9 (6115.5) | 7714.6 (6316.5) | 0.05 | 0.9 |
| Socioeconomic status 1 | 0.06 (0.9) | 0.5 (0.9) | 2.2 | 0.03 |
| Socioeconomic status 2 | 0.21 (0.9) | 0.26 (0.9) | 0.3 | 0.8 |

In the bivariate analyses, correlations between SED and variables of interest ranged from -0.16 to 0.70 (Table 8.5).

Table 8.5 Correlation coefficients for the relationship between variables with SED

| Variable | r (95% CI) | df | P |
|---|---|---|---|
| TEMIS ambient UV | 0.30 (0.16, 0.42) | 194 | $1.7 \times 10^{-5}$ |
| Skin exposed (weighted) | 0.70 (0.62, 0.77) | 193 | $2.2 \times 10^{-16}$ |
| SES principal component 1 | -0.11 (-0.27, 0.06) | 136 | 0.21 |
| SES principal component 2 | 0.19 (0.03, 0.35) | 136 | 0.02 |
| IPAQ: | | | |
| Work | 0.29 (0.16 0.41) | 193 | $3.7 \times 10^{-5}$ |
| Transportation | 0.14 (-0.00004, 0.27) | 193 | 0.05 |
| Domestic | 0.04 (-0.09 0.18) | 193 | 0.53 |
| Leisure | 0.07 (-0.07, 0.20) | 193 | 0.35 |
| Walking | 0.20 (0.06, 0.33) | 193 | 0.005 |
| Moderate MET-mins per week | 0.16 (0.02, 0.30) | 193 | 0.02 |
| Vigorous MET-mins per week | 0.28 (0.14, 0.40) | 193 | $8.7 \times 10^{-5}$ |
| Total score | 0.28 (0.14, 0.40) | 193 | $6.9 \times 10^{-5}$ |
| Total steps | 0.38 (0.19, 0.54) | 94 | 0.0002 |
| Weight | 0.10 (-0.04, 0.24) | 193 | 0.16 |
| Waist | 0.02 (-0.12, 0.16) | 193 | 0.76 |
| Hip | -0.09 (-0.23, 0.05) | 193 | 0.20 |
| % body fat | -0.16 (-0.30, -0.02) | 193 | 0.03 |
| BMI | -0.04 (-0.18, 0.09) | 193 | 0.54 |

Variables skin exposed (weighted), SES principal component 2, IPAQ work and walking domains, moderate and vigorous MET-mins per week, the IPAQ total score, and pedometer total steps, were significantly and positively correlated with SED. Body fat percentage was significantly and negatively correlated with SED. No other correlations were significant.

The t-tests revealed that males had higher SED than females (male 6.0 (4.3), female 3.4 (2.9), $t(114)=-3.6$, $p=0.0005$). Working outdoors was also associated with higher SED (working indoors 4.4 (3.4), working outdoors 7.5 (4.5), $t(24)=3.1$, $p=0.005$). Participants who were retired and working did not significantly differ in SED (working

4.5 (3.4), retired 5.7 (5.0), $t(43)=-1.5$, $p=0.1$). Finally we ran an ANOVA to examine the relationship between skin type and SED. This association was not significant ($F_{3,185} = 1.22$, $p = 0.30$).

To explore determinants of SED in Shetland we ran two multivariable regression analyses. The first (Model 1) used questionnaire-derived physical activity data from the sample who had returned full data (132 participants). The second (Model 2) used a subset of these 132 participants, who were the 64 participants who had worn a pedometer. Model 2 therefore included pedometer-derived step-count data and responses to the physical activity questionnaire. The models produced slightly different results.

Model 1 results are presented in Table 8.6. TEMIS ambient UV was significantly associated with a larger SED (Figure 8.6). There was a significant association with age, such that older individuals had a higher SED (Figure 8.7). Working outdoors was also significantly associated with higher SED (Figure 8.8).

Table 8.6 Results of linear regression including questionnaire-derived physical activity data, with square root transformed SED as the outcome and z-scored predictor variables

| | **Multivariable model (n=132)** | |
|---|---|---|
| **Predictors** | Est (95% CI) | p-value |
| Intercept | 2.03 (1.41, 2.67) | $2.8 \times 10^{-9}$ |
| TEMIS ambient UV | 0.21 (0.10, 0.34) | 0.0004 |
| Age | 0.19 (0.07, 0.31) | 0.002 |
| Indoor occupation | -0.44 (-0.96, -0.02) | 0.04 |
| IPAQ total score | 0.11 (-0.02, 0.22) | 0.09 |
| Fitzpatrick skin phenotype | | |
| Skin type 3 | 0.28 (-0.21, 0.77) | 0.27 |
| Skin type 4 | 0.36 (-0.09, 0.82) | 0.12 |
| Skin type 5 | 0.50 (-0.22, 1.01) | 0.06 |
| Skin exposed (weighted) | 0.08 (-0.02, 0.20) | 0.13 |
| Sex (male) | 0.15 (-0.16, 0.47) | 0.34 |
| Socioeconomic status 2 | 0.03 (-0.08, 0.15) | 0.57 |
| Body fat % | 0.04 (-0.10, 0.18) | 0.59 |

$R^2 = 0.34$; adjusted $R^2 = 0.28$

Figure 8.6 Scatterplot of the association between TEMIS ambient UVB and SED, Model 1

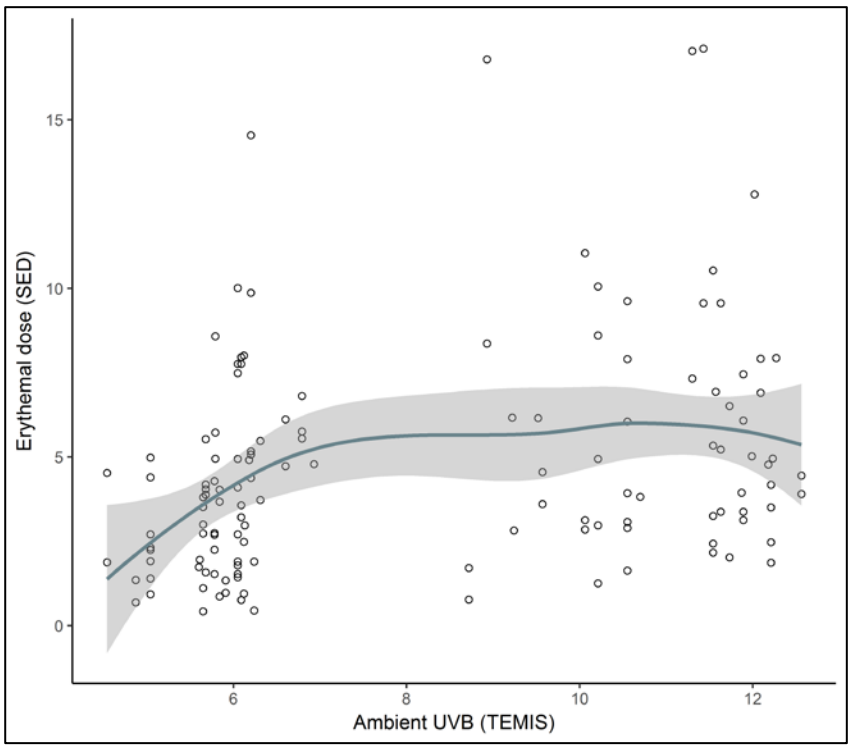


Figure 8.7 Mean SED by decade of age, Model 1

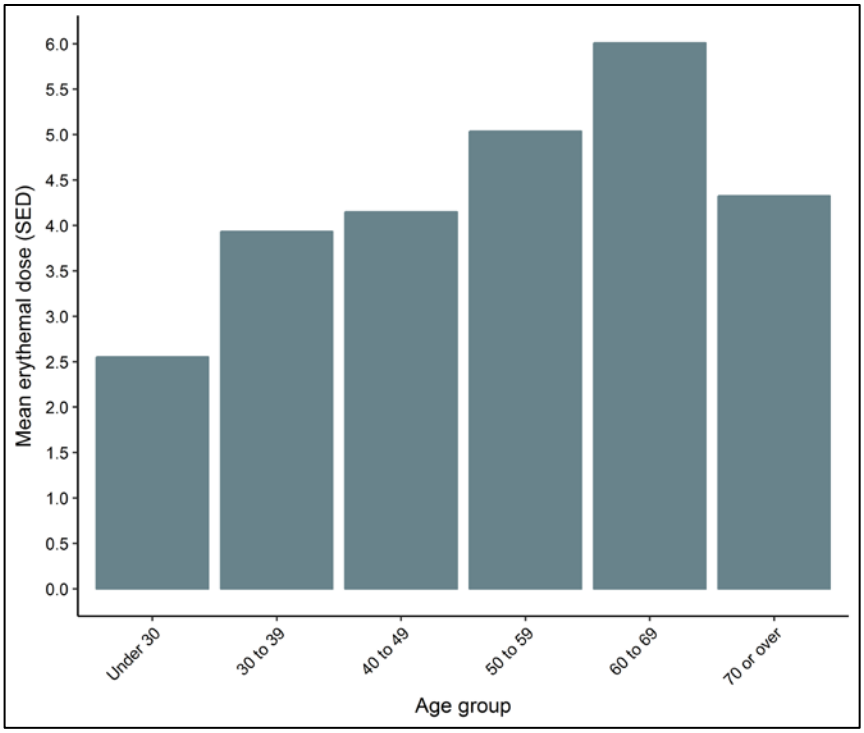
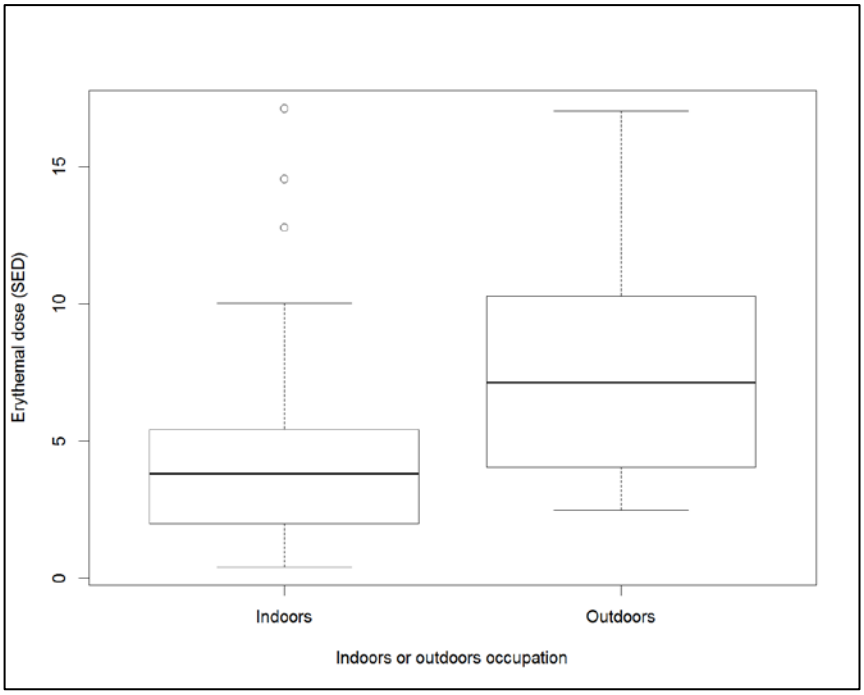



Figure 8.8 Boxplot of the association between occupation type and SED, Model 1



Model 2 results are presented in Table 8.7. Again, ambient UV and older age are both associated with higher SED (Figures 8.9 and 8.10). Total steps taken was also associated with a higher SED (Figure 8.11).

Table 8.7 Results of linear regression including pedometer-derived physical activity data, with square root transformed SED as the outcome and z-scored predictor variables

| | **Multivariable model (n=64)** | |
|---|---|---|
| **Predictors** | **Est (95% CI)** | **p-value** |
| Intercept | 1.90 (1.12, 2.69) | $1.1 \times 10^{-5}$ |
| Total steps (pedometer) | 0.37 (0.21, 0.52) | $1.3 \times 10^{-5}$ |
| Age | 0.21 (0.05, 0.38) | 0.01 |
| TEMIS ambient UV | 0.18 (0.003, 0.32) | 0.02 |
| Body fat % | 0.14 (-0.05, 0.34) | 0.14 |
| Sex (male) | 0.31 (-0.16, 0.78) | 0.19 |
| Fitzpatrick skin phenotype | | |
| Skin type 3 | 0.14 (-0.57, 0.86) | 0.68 |
| Skin type 4 | 0.26 (-0.47, 0.99) | 0.47 |
| Skin type 5 | 0.39 (-0.45, 1.22) | 0.35 |
| Socio-economic status 2 | 0.05 (-0.10, 0.2) | 0.48 |
| Indoor occupation | -0.19 (-0.74, 0.35) | 0.48 |
| IPAQ total score | 0.02 (-0.12, 0.18) | 0.77 |
| Skin exposed (weighted) | 0.004 (-0.14, 0.15) | 0.95 |

$R^2 = 0.57$; adjusted $R^2 = 0.47$

Figure 8.9 Scatterplot of the association between TEMIS ambient UVB and SED, Model 2

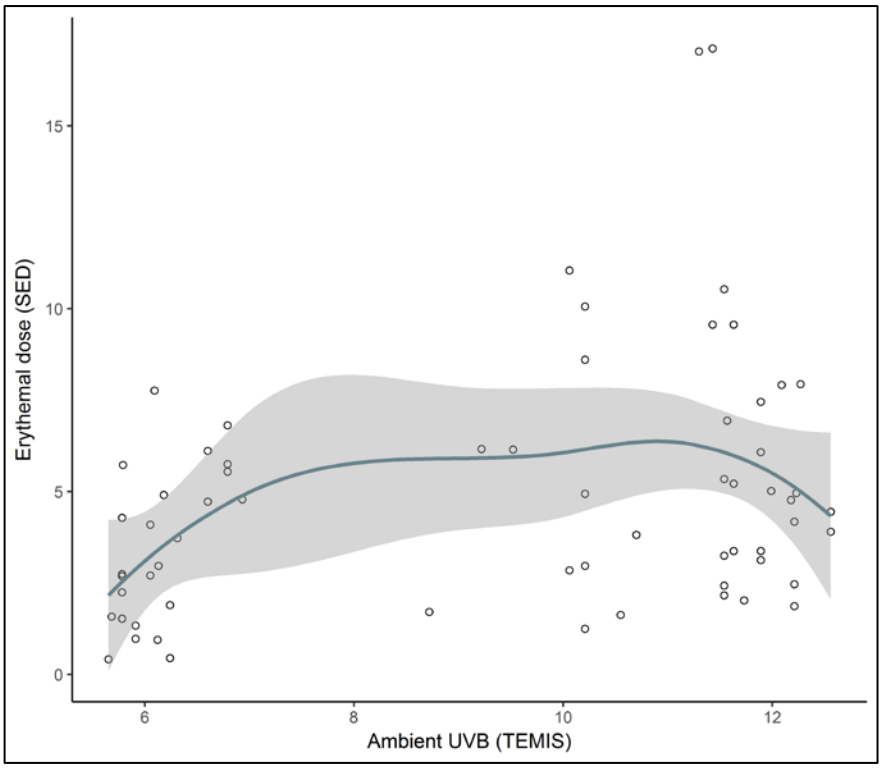



Figure 8.10 Mean SED by decade of age, Model 2

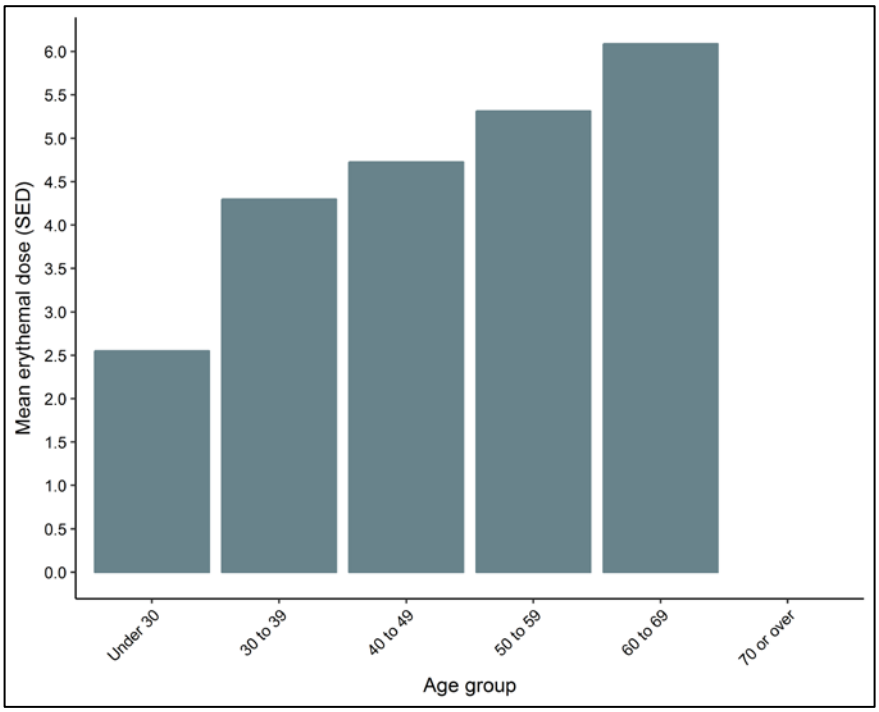
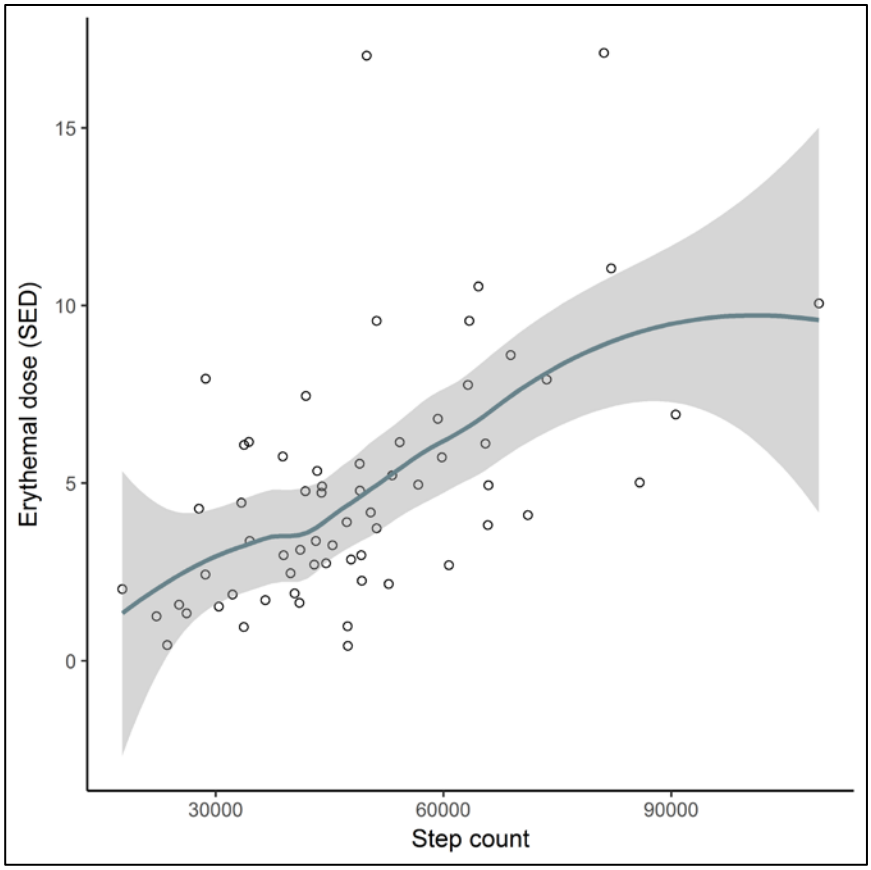


Figure 8.11 Scatterplot of the association between step count and SED, Model 2




We examined why working outdoors was associated with higher SED in Model 1, but not in Model 2. Of the 132 people in Model 1, 16 people were classified as mainly working outdoors. Of these 16 people, 11 had pedometers and were therefore counted within the 64 people in Model 2. It seems that, in Model 2, a higher step-count might have been a proxy for working outdoors.

Finally, we examined several traits, including the sex, occupation, and physical activity of individuals who had very high UV readings, as these individuals may be informative about what contributes to greater UV exposure. The 10 highest UV badge readings ranged from 13.2 to 22.0 SED. These participants were aged 50 or over, were predominantly male, and several worked outdoors (Table 8.8). A chi-square test revealed that older people are no more likely than younger people to have an outdoor

occupation ($\chi^2(3, N = 132) = 1.04$, $p = 0.79$); however, within our dataset, significantly more men than women work in outdoor occupations ($\chi^2(1, N = 132) = 18.14$, $p < 0.01$). The two individuals in Table 8.8 who work mainly indoors (teachers) were on school holidays, and their data collection week coincided with favourable weather.

Following this, we graphed the rainfall, sunshine, and TEMIS ambient UV for each week throughout the study period (Figure 8.12). As expected, ambient UV decreased throughout August; however, participants with high UV scores tended to have their data collected over weeks during which there was more sunshine and less rainfall. An exception to this is one participant whose data collection week commenced on 6 August 2014. According to weather data (Figure 8.12), this was a week of good sunshine but also very high rainfall. Closer inspection of daily data (not shown) found that the majority of the rain fell over the final four days; the first three days of the week had been characterised by low rainfall and sunshine (Online, 2014).

Table 8.8 High UV readings and individual information

| Study w/c | Sex | Age | Occupation | Pedometer score | IPAQ score | UV badge (SED) | Other comments |
|---|---|---|---|---|---|---|---|
| 25 June | M | 64.8 | Disabled/retired | 31817 | 810 | 15.1 | - |
| 1 Jul | M | 51.3 | Builder | 45751 | 16170 | 22.0 | - |
| 2 Jul | M | 62.9 | Builder | 49845 | 13194 | 17.0 | - |
| 10 Jul | M | 62.8 | Builder | NA | 14100 | 12.8 | - |
| 17 Jul | M | 50.5 | Teacher* | 81140 | 14130 | 17.1 | - |
| 21 Jul | F | 51.4 | Teacher* | 72572 | 5145 | 19.9 | Participant says very sunny week and spent much time sunbathing |
| 1 Aug | M | 51.7 | Fisherman/crofter | NA | 6840 | 16.8 | - |
| 6 Aug | M | 54.0 | Fish farmer | 26294 | 13182 | 15.5 | - |
| 22 Aug | F | 67.9 | Retired | NA | 13638 | 14.5 | - |
| 27 Aug | M | 71.8 | Retired | NA | 17448 | 13.2 | - |

*Schools broke up for summer on Friday 4th July 2014, so these dates were in the summer holidays when teachers were not in school

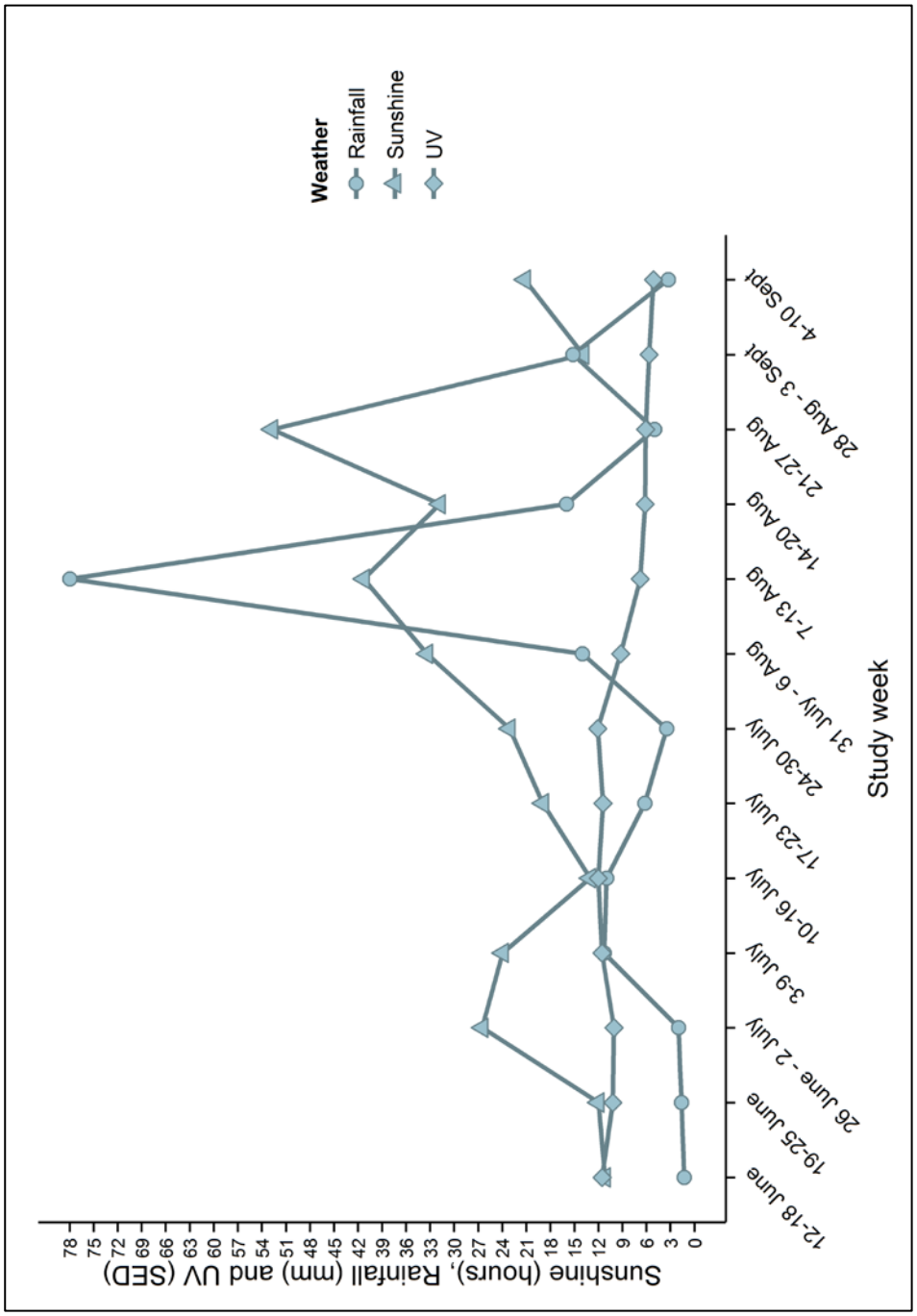



Figure 8.12 Sunshine hours, rainfall and ambient UV by week over the study period

## 8.4 Discussion

We aimed to understand how different variables are related to UV exposure, and to identify determinants of UV exposure, in Shetland. To do so, we ran a series of bivariate analyses with individual UV exposure as the outcome. We then ran two multivariable models, including all participants with complete data in Model 1, and the subset of participants who had been supplied with a pedometer in Model 2. All analyses conducted for this chapter were exploratory; that is, they were not seeking to test any hypotheses that had been specified *a priori*. As such, we did not adjust for multiple testing. However, any confirmatory studies that use multiple tests would require a more stringent p-value.

In Shetland, we found that individual UV exposure is generally low, as may be expected in a county situated at such a high latitude. The highest weekly exposure was 22 SED. Diffey (2002b) suggested that an SED of 4 on previously unexposed skin should cause moderate erythema. The mean exposure for each day would therefore have been insufficient to produce moderate erythema. However, it is unlikely that UV exposure was evenly spread across the study days – the weather does not follow such a consistent pattern. On some days the exposure could have resulted in erythema whilst on other days it would not. Such fluctuation in exposure could have health implications for the population of Shetland.

We found that older participants had greater UV exposure than younger participants in both multivariable models. Furthermore, in Model 1, we observed that working indoors was associated with reduced UV exposure. Indoor working was not associated with reduced UV exposure in Model 2; however, the total pedometer steps was associated with higher UV exposure. Further examination of these data suggested that, because many pedometer-wearers in Model 2 were also outdoor workers, total steps taken was a proxy for outdoor employment.

Similar patterns involving age and outdoor workers have been observed elsewhere. A prospective observational study found that, in Denmark, individuals aged younger than 20 years had significantly more sun exposure per year. However, gardeners received the most UV exposure on work days (Thieden et al., 2004). Another study found that, in Thailand and South Korea, where sunshine is abundant, older adults have higher vitamin D levels. Older adults, who are more likely to be retired, also have increased amounts of leisure time to spend outdoors (Nimitphong and Holick, 2013). Additionally, the changing economy has meant that whilst older people tend to work in traditional occupations which are often outdoors, younger adults are pursuing office-based careers which reduces their sun exposure and therefore vitamin D levels (Choi et al., 2011; Chailurkit et al., 2011). We observed a similar pattern in Orkney where outdoor workers had significantly better vitamin D than indoor workers. Like in Thailand and South Korea, Orcadians with better vitamin D tended to be older and had increased leisure time for sun-seeking (Weiss et al., 2016) (Chapter 7). One study from the United States found no association with age and UV exposure, however this study was limited to adults aged 40 and over (Cahoon et al., 2013). The positive effect of age with UV exposure in Shetland therefore complements findings from elsewhere; however, as older people are no more likely to have an outdoor occupation than younger people in our cohort, this effect is probably not related to outdoor occupations in Shetland.

Total steps taken was the only physical activity variable that was associated with greater UV exposure. It is interesting that no other physical activity variable significantly contributed to UV exposure, particularly as physical activity has been linked to an increased risk of sunburn (Jardine et al., 2012) and non-melanoma (Schnohr et al., 2005) in both northern and southern hemispheres. It is therefore

reasonable to assume that physical activity is often associated with greater sun exposure.

In bivariate analyses, the physical activity variable most strongly correlated with UV exposure was total steps taken. Several IPAQ domains were also significantly correlated with UV exposure, but the correlations were weaker. The IPAQ data that were most strongly correlated with UV exposure were the work domain, vigorous MET minutes per week, and the total IPAQ score. However, the IPAQ data comprise many forms of physical activity and do not differentiate between indoor or outdoor activity. Greater indoor activity, or activity outwith daylight hours, would lead to these smaller correlations between activity and UV exposure. IPAQ data also relies on recall data for the seven days preceding questionnaire completion, which may not be as accurate in describing exercise taken. There is also no scope for defining the period during the day or week during which exercise was taken. The pedometer data are instead collected at the time of activity and do not rely on recall. It could be argued that, within daily life, people are more likely to have a higher step count when they are outside and are more likely to be outside in better weather – making the most of pleasant weather in a county characterised by overcast skies. It is therefore possible that total steps taken is the best indicator of physical activity in favourable weather when UV is plentiful.

The strongest of all the correlations with UV exposure was the skin exposed (weighted) variable. However, this variable was not significant in either multivariable model. The way in which skin exposed (weighted) was constructed may mean that it acts as a proxy for UV exposure. A high score for skin exposed (weighted) indicates that fewer clothes were worn and more time (or periods in the middle of the day) was spent outside, and it is therefore likely to reflect warmer and sunnier weather when UV would be more abundant. It is, however, interesting to note that satellite-derived

ambient UVB was only moderately correlated with individual UV exposure, but was significantly associated with greater UV exposure in both models. Ambient UV was previously identified as a strong predictor of individual UV exposure in a US cohort (Cahoon et al., 2013).

We found no difference in UV exposure between men and women in either multivariable model. Other studies also did not find a difference between men and women. One study found that there was no significant difference in UV exposure between boys and girls in England on weekdays, although boys received significantly more UV at weekends (Diffey et al., 1996). A study from Denmark found that there was no difference in UV exposure between men and women, although girls received a significantly higher UV dose than boys (Thieden et al., 2004). However, a US study found that men received a significantly higher UV dose than women (Cahoon et al., 2013). Although we found no significant sex differences in either model, the t-test showed that males were higher in UV exposure. However, in our data we also found that men were significantly more likely to work outdoors.

It is important to note that directly comparing our results with other studies is complicated by several factors, including the anatomical placement of the dosimeter. Other studies have asked participants to wear UV badges on their wrist (Thieden et al., 2004; Thieden et al., 2005b; Thieden et al., 2005a), shoulder (Kimlin et al., 1998a; Chodick et al., 2008; Cahoon et al., 2013), chest (pinned to clothing or on a necklace) (Diffey et al., 1996), and multiple anatomical sites (Herlihy et al., 1994). These differences in the anatomical placement of the dosimeter can lead to differences in UV exposure measurement (Diffey et al., 1977). Furthermore, different types of dosimeter have been used to collect UV exposure data, including, among others, polysulphone film (Diffey et al., 1996; Kimlin et al., 1998a; Herlihy et al., 1994; Cahoon et al., 2013) and

electronic dosimeters (Thieden et al., 2004; Thieden et al., 2005b; Thieden et al., 2005a; Thieden et al., 2006). Therefore, although patterns can be observed within the literature, it is difficult to directly compare studies with substantially different methodologies.

Additionally, there is little literature assessing the repeatability of polysulphone to measure individual UV exposure. Repeatability requires that, for a sample, two measurements are made under identical conditions on each subject (National Institute for Standards and Technology, 1994). Reliability can then be assessed by relating the extent of within-subject measurement error to between-subject measurement error. Where reliability is high, within-subject variability is small compared to between-subject variability (Bartlett and Frost, 2008). To achieve identical conditions for the measurement of UV using polysulphone, the simultaneous wearing of two or more badges at the same body site would be necessary. Although studies have been undertaken to understand how different anatomical placement of badges can result in different levels of UV measurement, the actual repeatability of the UV exposure measure has not been assessed (Waterston et al., 2004). The reliability of how different dosimeters perform when measuring individual UV exposure has also not been explored. Such a study would be a type of reproducibility study. Reproducibility refers to variation in measurement made on the same subject, however one condition changes. In this case, it may be wearing two different types of UV dosimeter. It may be that each type of dosimeter yields different results. Such a study could be valuable in understanding the limitations and comparability of different methods used in different studies.

However, most studies that explore individual UV exposure attempt to understand how dosimeter readings correlate with other UV measures, such as

ambient UV (Cahoon et al., 2013), or sun exposure diaries (Chodick et al., 2008; Køster et al., 2017). Although the present study showed a weak correlation with ambient UV, there was a strong correlation with skin exposed (weighted) which, as described earlier, took into account length of time and periods of the day spent outside. We did not, however, explore the repeatability of polysulphone film.

There were further limitations to our study. One limitation of polysulphone film is that it provides a cumulative measure of UV exposure which cannot be broken down into smaller units of time, whilst electronic dosimeters have enabled UV exposure to be time-stamped. The cumulative measure therefore depends on the length of time for which the polysulphone was worn. Polysulphone studies have been carried out over two days (Kimlin et al., 1998a; Herlihy et al., 1994), seven days (Cahoon et al., 2013), and others have been over longer periods with different badges for weeks and weekends (Diffey et al., 1996). We provided participants with one badge for one week, which meant that we were also unable to ascertain differences in UV exposure behaviours between weekdays and weekends. However, by providing one badge only, we hoped to make the study easier for participants and to increase compliance.

We were also limited by the amount of time that we had in which to conduct the study and by the amount of funding available. Physical activity data can be gathered with much greater accuracy. However, at the time of the study, these methods were beyond our budget. We therefore used the combination of pedometers and IPAQ. It is important to note that, since the study was carried out, there have been advances in physical activity data collection. Wearable physical activity trackers which can synchronise to a smartphone app have substantially reduced in price and are likely to give a more accurate measure of activity.

Finally, we would have liked to measure vitamin D levels for all participants of the UV study, to understand how UV exposure in Shetland translates to blood vitamin D. However, again for reasons of time and funding, we were unable to do so.

## 8.5 Conclusion

Individual UV exposure was generally low in the summer months in Shetland. This could have consequences for vitamin D levels in the islanders and for their general health. We found that greater UV exposure was associated with ambient UV as well as total steps taken. Total steps could reflect working outdoors or taking advantage of favourable weather when it arrives. Indoor workers receive less UV exposure than outdoor workers; however as Shetland is a largely indoor-working archipelago, few people are able to take full advantage of periods of sunshine. We found that younger people get less UV exposure than older people in Shetland, a county with a high prevalence of MS. This finding is consistent with other literature of both countries with abundant sunshine and other high-latitude countries. Like the findings of our Orkney vitamin D study, the reduced exposure to UVB in younger people who are within their child-bearing years could have consequences for the autoimmune health of offspring, as well for the individuals who are within the peak years for MS diagnosis. Further research to understand how UV exposure translates into vitamin D levels in this population would help to understand the health implications of both UV exposure and vitamin D.

# CHAPTER 9. CONCLUSIONS

A history of transient neurological disease, characterised by intermittent paralysis that customarily results in a final decline is well-recorded in diaries, personal correspondence, medical notes, and literature dating from medieval times. However, this collection of symptoms that can indicate MS were only recognised as a disease towards the end of the nineteenth century. Since that time, diagnostic criteria have become increasingly sophisticated as our understanding of the biological and molecular processes that are characteristic of MS has grown. However, different presentations and symptoms of MS, as well as differential diagnoses that require exclusion, can result in a difficult and sometimes lengthy diagnostic procedure.

Alongside these advances, several factors have been identified that are associated with increased risk of MS. A higher frequency of MS noted in families of affected individuals led to the suggestion of genetic involvement. GWAS have since identified genetic variants, each with a modest effect. Variants at the *HLA* locus confer the strongest genetic effects. Environmental risk factors, which have fairly consistently been associated with increased MS risk, include EBV infection and smoking. Many ecological studies have also identified an association between higher latitudes and greater MS prevalence. This finding led to the formulation of a theory whereby reduced UV exposure at higher latitudes leads to vitamin D deficiency, and results in an increased risk of MS.

The high prevalence of MS in Orkney and Shetland was first observed in the 1950s (Sutherland, 1956). Subsequent studies continued to find a high prevalence of MS in the Northern Isles, with Orkney most affected. However, despite several researchers conducting studies into MS in these archipelagos, mainly in the 1970s and early 1980s, the search for possible reasons for the high prevalence has not proved fruitful. The isolated geographical location and characteristic inclement weather in

Orkney and Shetland has historically meant that travel to and from the islands has been limited, resulting in genetic isolation. Such isolation may have consequences for health. Previous work in Orkney and Shetland explored genetic effects and MS (Roberts et al., 1979; Roberts et al., 1983; Roberts, 1991), and the possible involvement of environmental exposures (Poskanzer et al., 1981). However, these studies did not set out to investigate two pertinent risk factors resulting from the high-latitude geographical location and often overcast weather of Orkney and Shetland: reduced UV exposure and vitamin D deficiency.

This thesis aimed to explore risk factors for MS in Orkney and Shetland that may help to explain the high prevalence. I followed two avenues of research. The first comprised potential adverse health consequences resulting from genetic isolation, which may lead to a greater risk of MS. These explorations used datasets of MS cases collected in the 1970s and in recent prevalence and genetic epidemiological studies (Poskanzer et al., 1980a; Visser et al., 2012; McQuillan et al., 2008). The second involved potential health consequences from the high-latitude geographical location of Orkney and Shetland. These analyses comprised data from recent genetic epidemiological studies and from data collected for this thesis in cohorts of people without MS.

## 9.1 Findings and limitations

I began by conducting analyses using MS cases in Orkney and Shetland alongside data from both recent and historic datasets. My three aims were to: 1) to understand whether MS cases cluster more frequently in particular parishes or isles than expected by chance; 2) to calculate heritability estimates of MS in Orkney and in Shetland, and 3) to explore whether particular parishes or isles contribute more to the gene pool of MS in Orkney and Shetland than do others.

Clustering studies have been used to identify possible causes for disease by detecting an excess of cases against a background rate. These types of studies are often used to identify an environmental aetiology, such as polluted water sources, exposure to heavy metals, or risks associated with certain occupations. However, in such investigations, studies of diseases with a short period of latency between exposure and disease onset are less complicated to conduct than diseases such as MS, where any environmental cause may precede disease onset or diagnosis by many years. Many studies in multiple geographical areas have attempted to elucidate potential causes for apparent clusters of MS, but none have been fruitful. Clustering studies can furthermore be hindered by problems, including how to define disease and how to define the population at risk. These two factors were of the greatest concern in our analyses (Chapter 4). With incomplete case ascertainment, an inaccurate population-at-risk, and no information regarding possible exposures between birth and disease onset, the results of our study must be interpreted very cautiously. There is a strong possibility that, because of these problems, the aggregations of disease that I observed are artefacts rather than disease clusters; additionally, there are multiple plausible reasons for any apparent clusters that could not be excluded by these data.

Several studies have estimated the heritability of MS. Using twin studies, these heritability estimates have ranged from modest (0.15 (95% CI 0.0, 0.77)) to substantial (0.64 (95% CI 0.36-0.76)) (Table 5.1). I estimated the heritability of liability to disease using parent-offspring trios, and obtained estimates of heritability that were consistent with the literature (Chapter 5). However, despite the high prevalence of MS in Orkney and Shetland, and the inclusion of all cases about which I had a good degree of certainty, the sample sizes were small with few parent-offspring cases. This meant that the confidence intervals were wide and crossed zero, leading to a high degree of uncertainty in the estimates. However, despite such difficulties, these results show that

there is a genetic component to MS in Orkney and Shetland, although probably no more than is found elsewhere.

My further investigations into the possibility that some parishes or isles may have contributed more to the MS gene pool than others were also hindered by the small sample sizes, which led to overlapping error bars in each district and therefore ambiguity in interpreting results. This limitation meant that it was not possible to conclude that genes arising from any district in the archipelagos were more responsible for the high prevalence of MS than those from other districts; however, neither was it possible to rule this out.

In the second half of the thesis I explored environmental risk factors using cohorts based in Orkney, Shetland, and mainland Scotland, comprising people who do not have MS. These two cross-sectional studies explored vitamin D in Orkney and UV exposure in Shetland, respectively.

Contrary to what was expected, I found that people in Orkney had higher mean vitamin D than people in mainland Scotland (Chapter 7). On closer investigation, I found that, although levels of vitamin D were low in both cohorts, Orkney had a smaller proportion of people who were severely deficient. It was this difference that led to the higher mean vitamin D in Orkney.

I also found that, contrary to previous findings, older people in Orkney had higher vitamin D levels than younger people. There is considerable evidence that supports a decline in vitamin D levels with increasing age. Furthermore, there is a biological reason for this effect, as our ability to synthesise vitamin D decreases as we get older. However, certain behaviours can result in the opposite effect. In Orkney, this effect occurred because people in older age groups were more likely to work outdoors,

and to take foreign holidays. Furthermore, although I did not have data to support this theory, I suspect that older people are less likely to have work and schooling restrictions to prevent them from taking foreign holidays at times of year when there is no possibility of sunshine-induced vitamin D synthesis in Orkney. However, what this means is that the younger generation, who are more likely to work indoors and miss any benefits of Orkney sunshine, and are less likely to holiday abroad, are at increased risk of vitamin D deficiency. This deficiency comes during a period of life in which MS is most likely to be diagnosed, and when pregnancies are most likely to occur, potentially conferring risk to the unborn child.

My investigations into individual UV exposure in Shetland found a similar pattern to that observed in Orkney. Individual UV exposure was generally low, even in the summer months (Chapter 8). Complementing previous literature, I found that greater ambient UV, and also the total number of steps taken measured by pedometers, were associated with greater UV exposure. The total steps variable likely acts in two ways: firstly, it appears to be a proxy for working outdoors where, like the farming cohort in Orkney, the occasional vitamin D-strength sunshine is maximised. Secondly, total steps probably reflects better weather. This is because people are more likely to walk further (leading to a greater step-count) when they are outside, and are more likely to be outside when the weather is favourable. Also, like the Orkney vitamin D study, I found that older people were more likely to receive more UV exposure than younger people in Shetland. Quite how that translates to vitamin D levels is unknown; however, it is concerning that the younger population are, again, more vulnerable to low levels of a modifiable risk factor during a possibly critical period of their lives, in terms of MS risk.

## 9.2 Future Steps

There are several ways that this work can be carried forward. To begin, I will discuss in broad terms the direction that future research could take which has been noted throughout this thesis. I will then focus on the direction that studies conducted in Orkney and Shetland could follow.

Firstly, the scoping review highlighted how few intervention studies have been undertaken, particularly over periods of time long enough, and with doses of vitamin D high enough, to achieve a biological – and potentially beneficial – effect. As such, the literature does not currently provide evidence of a causal link between vitamin D and MS onset and/or progression. However, the scoping review did reveal some promising intervention studies with results regarding vitamin D supplementation and slower progression of MS, measured by both disability score and by MRI-identified lesions. Furthermore, one study found that vitamin D may suppress conversion from clinically isolated syndrome to MS, which deserves further exploration and attempts at replication.

More intervention studies into the effects of vitamin D on MS risk and progression are warranted. This need is all the more keenly felt when placed alongside evidence from Mendelian randomisation studies, which show an increased risk of MS with lifetime vitamin D deficiency. Further trials comprising adequate vitamin D supplementation over a long enough period to observe a reliable effect could produce fruitful results in understanding whether vitamin D has the ability to prevent or delay MS onset, and whether it has a therapeutic effect in established MS. Such findings could be incredibly valuable and beneficial in both protecting susceptible populations and in treating those affected.

Secondly, the scoping review highlighted that the literature exploring the role of UV exposure in MS is scarce; compared to the vitamin D literature, UV exposure is under-researched. The literature that does exist is heterogeneous with diverse findings. There is undoubtedly scope to further explore the role of UV exposure in MS risk and disease progression, both as a determinant, and independent, of vitamin D. Additionally, it would be beneficial to examine whether gestational or childhood exposure is crucial, particularly as this may lead to the ability to target interventions towards pregnant women or children, or whether lifetime UV exposure is as important in any mitigation of MS risk.

Thirdly, the Viking UV study highlighted that the repeatability and reproducibility of UV dosimeter measures have been little explored. This gap could provide an opportunity to explore how reliable the measures obtained by using such dosimeters are, and also how studies that use different methods can be reconciled.

Looking specifically at the Viking UV study in Shetland, it is important to understand how UV exposure translates into vitamin D levels at such high latitudes, and how these compare to Orkney. Ensuring comparability of the blood samples with ORCADES may however be difficult, particularly as the laboratory used for both SOCCS and ORCADES has changed their methods of blood vitamin D analysis in the interim. This limitation should be noted in future studies that attempt to compare Shetland vitamin D measures with Orkney. Similarly, there is ongoing development of more accurate ways in which to measure physical activity and UV exposure. Should a more detailed follow-up study be commenced, it would be advantageous to use these more detailed methods, such as electronic time-stamped dosimeters, which provide a more accurate account of sun exposure over different periods of time, including weekdays and weekends. Furthermore, incorporating other relevant factors, such as diet, could

help to understand if the lower MS prevalence in Shetland compared to Orkney could be attributed to intake of oily fish, as it has elsewhere at similar latitudes (Bäärnhielm et al., 2014). More detailed dietary data, as well as measurement of individual UV exposure in Orkney could also help to further understand the pattern of vitamin D noted in the Orkney Vitamin D Study. Incorporating such data into analyses would help to form a clearer picture of the determinants of UV exposure and vitamin D in both archipelagos.

Finally, although the limitations inherently associated with clustering analyses, alongside the available data, mean that there is no positive way to move forward that could increase clarity, there are ways in which the heritability of MS could be further explored. It was clear that my studies of the genetic contribution to MS in Orkney and Shetland using genealogical data did not produce reliable results; however, today there are methods for measuring heritability genomically. Pedigree analyses are limited by the size and accuracy of the pedigrees on which they are based; however, marker-based approaches in which relatedness is estimated can result in less biased and more precise estimates. Similarly, explorations of genomic data may provide clarity regarding the genetic contribution to disease in these archipelagos, including the presence and frequency of rare and deleterious mutations, which may act to increase MS risk in these genetic isolates.

## 9.3 Policy and Public Health Implications

It is of concern that both studies found that vitamin D deficiency and reduced UV radiation are more frequently observed in the younger generations in these islands. With changing lifestyles in which people are increasingly working indoors, for example in office environments, over traditional, outdoor occupations, such as farming, the prevalence of these risk factors is only likely to increase. It should be remembered that adequate UV exposure and optimal vitamin D status, as well as being modifiable risk

factors for MS, are important for protection against other diseases including bone conditions and some cancers.

That said, offering advice regarding vitamin D supplementation is a thorny issue. As described in the scoping review, it remains unclear how much vitamin D should be taken, and for how long, to have a clinically beneficial effect. However, vitamin D is a relatively safe and well-tolerated supplement, which appears to have multiple favourable effects for health. I myself have not hesitated to take a daily supplement and will continue to do so, particularly while residing in a northerly latitude. As $D_3$ appears to be more biologically active and better at raising blood vitamin D levels than $D_2$, it would seem that $D_3$ or a combination of $D_2$ and $D_3$ would be advisable, and, depending upon age and circumstances, from 400IU per day (Holick et al., 2011). Such an amount is unlikely to be harmful and the correct level of supplementation may be helpful. However, a vitamin D-rich diet, including oily fish, eggs, red meat, and fortified foods such as breakfast cereals and some milk and juices may also have a beneficial and healthful impact.

Vitamin D from sunlight enters the circulatory system more quickly than that from dietary sources and, moreover, carries no risk of toxicity. However, attempting to provide advice about obtaining vitamin D from sunshine is fraught with difficulties resulting from the myriad personal and environmental factors that can affect UV-induced vitamin D synthesis. There is no one-size solution for every individual and therefore any such exposure should be undertaken judiciously, taking care not to burn. Moderation is key; however because strong sunshine is in short supply in these high-latitude islands it could be difficult to achieve moderate levels of exposure on a normal day, and a danger of over-exposure on occasional bright, strongly-lit days, which could result in harm.

There is an argument that a modifiable risk factor, such as vitamin D deficiency, should be addressed generally by fortification of dairy products (as it is in the US), and particularly in at-risk communities by routine and regular supplementation. However, there is currently not the weight of evidence to support such a programme. If further research were able to establish a causal connection between vitamin D deficiency and MS, there could be little argument for a swift translation into policy to attempt to modify these risk factors in at-risk communities. It must be hoped that communities such as Orkney and Shetland could benefit from such research and subsequent policy implementation, in an effort to reduce MS and its effects, and to protect a vulnerable population from a devastating, and all too prevalent, disease.

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## Appendix A: Additional information for Table 4.2

Six cases were identified within 500 yards of each other in Berkshire, England. Following an earlier hypothesis (Cone et al., 1934), this cluster was attributed to the high lead content of the soil and water. Later research, however, failed to identify any increased risk in people who worked with lead or in high-lead areas (Campbell et al., 1950).

Fourteen cases were identified in the 10,000-strong population of Mansfield, Massachusetts. The town water supply had been polluted some years prior to this cluster, and was identified as a possible aetiological factor (Eastman et al., 1973). However, as only eight of these fourteen cases had lived near the polluted water supply, and one had not been born during the period of pollution, chance, or other unrecognised agents, may have caused this cluster.

Possible occupational clusters of MS have also been investigated. In the 1940s an apparent cluster of four MS cases among people working with lamb swayback, a demyelinating disease in sheep resulting from copper deficiency, was observed. However, MS was also present in regions where there was no swayback, and there was no demonstrable effect of copper deficiency on MS (Campbell et al., 1947; Dean et al., 1985).

Further, research on a cluster of MS in Key West, Florida noted that seven of the nineteen definite cases were nurses who worked at the same hospital. However, a later study revealed there was no increased risk to medical personnel of working with MS patients (Dean and Gray, 1990). A potential hazard close to the hospital was noted: a landfill site that oozed red vermillion and regularly caught fire (Ingalls, 1986a). Although no causal agent was identified, the author hypothesised that the vermillion

ooze was from ships' paint and contained mercury, and that burning sent hazardous material into the air.

Six cases in two families were observed in the 450-strong population of Mossyrock, Washington. Koch et al. (1974) postulated that this cluster was associated with a possible but unconfirmed smallpox epidemic fifty years prior. However in later years a further unconfirmed theory attributed the cluster to smelting cinnabar to obtain mercury (Ingalls, 1986a).

# Appendix B: Scoping Review Protocol

## Methods

### Framework stage 1: Identify a Research Question

A starting research question is - "A scoping review to map the literature concerning vitamin D, UV exposure and multiple sclerosis, to identify evidence for an association between MS onset, pathology and progression". This question is broad to be inclusive and reduce the risks of missing relevant articles, however it is anticipated that the development of the research question will be an iterative process, with increasingly refined questions emerging once the volume and extent of literature has been assessed (Arksey and O'Malley, 2005).

### Framework stage 2: Identifying Relevant Studies

To be as inclusive as possible, we will undertake several different searches involving electronic databases, hand-searching reference lists of key papers and searching any key journals identified as a result of earlier searches. We will further endeavour to identify grey literature.

For the reason of time and resources, searches will be limited to English-language publications.

#### *Eligibility criteria*

We will include observational study designs (cohort, case-control, cross-sectional) studies, experimental (randomised control trials (RCTs)) and genetic studies (Mendelian randomisation, genome-wide association studies (GWAS) and candidate gene studies) that examine the association between vitamin D, either 25(OH)D or 1,25(OH)D, or UV exposure, and MS defined according to the Poser (Poser et al., 1983) or McDonald (McDonald et al., 2001) criteria. Studies involving MS patients of all age groups will be considered. Animal studies using EAE as a model of MS will be included.

Ecological studies that do not use individual measures of either vitamin D or UV exposure will be excluded.

More detailed inclusion and exclusion criteria may be developed 'post-hoc' as familiarity with the type and focus of identified literature increases (Arksey and O'Malley, 2005). To be transparent, if feasible, excluded studies will be tabulated and presented in the final review with the reason for exclusion.

### *Databases*

We will search Medline, Embase, Biosis, Web of Science and the World Health Organisation (WHO) International Clinical Trials Registry Platform (ICTRP). We will search for grey literature through the Proquest Dissertations and Theses Global database (PQDT), and from a search of Google Scholar.

### *Search Strategy*

The following terms will be used to search for relevant papers. Boolean operators AND and OR will be used to link search terms. Where options exist, search terms will be exploded to include all variations of the term.

*Vitamin D OR*

*Cholecalciferol OR*

*Ergocalciferol OR*

*25-hydroxyvitamin D OR*

*1,25-dihydroxyvitamin D OR*

*Calcidiol/calcifediol OR*

*Calcitriol OR*

*25(OH)D OR*

*1,25(OH)2D OR*

*Ultra-violet radiation OR*

*Sunlight OR*

*Sun exposure OR*

*UV OR*

*UVB OR*

*UVR OR*

*Ultraviolet radiation OR*

*Heliotherapy OR*

*Phototherapy*

*AND*

*Multiple sclerosis OR*

*Relapsing Remitting Multiple Sclerosis OR*

*Primary Progressive Multiple Sclerosis OR*

*Neuromyelitis optica OR*

*Devic disease OR*

*Tranverse myelitis OR*

*Optic neuritis OR*

*Experimental autoimmune encephalomyelitis OR*

*EAE*

### Framework stage 3: Study Selection

Study selection will be an iterative process with refinement of the search strategies following assessment of the volume of identified literature. Eligibility criteria will be pilot-tested on a random sample of articles and refined accordingly. One investigator will assess all titles and abstracts against these refined eligibility criteria. A second investigator will assess 20% of title and abstracts against these eligibility criteria; levels of agreement will be evaluated and disagreement resolved by consensus. Study abstracts that do not meet eligibility criteria will be excluded. Where there is insufficient information in the abstract to reach an informed decision for inclusion, the

full text will be sourced. The full text will also be sourced for all studies that meet the inclusion criteria.

### Framework stage 4: Charting the Data

To form the evidence base of the scoping study and summarise work that has been undertaken to date, extracted data will be entered into a spreadsheet based on Arksey and O'Malley's recommendations (Arksey and O'Malley, 2005), including the following headings.

*Author, year of publication, study location*

*Intervention type and comparator (if any); duration of the intervention*

*Study populations*

*Aims of the study*

*Methodology*

*Outcome measures*

*Important results*

*Standardised measures (if any)*

This form will be tried on a small selection of papers and refined further so that data extracted will reflect the aims of this scoping study (Levac et al., 2010). One investigator will read each article and extract relevant data; a subset of papers will be checked by a second investigator. Study quality is not generally assessed in a scoping review as the aims are to map the literature and identify gaps in the evidence base, and to pinpoint areas for future systematic review (Arksey and O'Malley, 2005; Levac et al., 2010).

### Framework stage 5: Collating, Summarising and Reporting Results

Collating, summarising and reporting results will be tackled in three distinct steps as laid out by Levac et al. (Levac et al., 2010). Firstly, basic descriptive numerical analyses will be presented to summarise the literature in tables and charts mapping:

*The distribution of studies geographically*

*Range of interventions/study designs*

*Measures used*

*Research methods used*

Secondly, we anticipate that this process will identify themes in the literature around which the following review can be built under section headings to report the results. To ensure that a consistent approach in reporting findings is maintained, Arksey and O'Malley suggest the use of a template, firstly summarising the characteristics of papers included in each section, and then a series of headings to structure the commentary around. These headings may include, depending on the section, interventions, sample sizes, participants, methods, outcomes, studies from world regions, and gaps remaining in the research (Arksey and O'Malley, 2005).

This consistent approach should give a comprehensive overview of the existing literature, allow comparisons across different studies and identify gaps in the evidence base (Arksey and O'Malley, 2005). It should also enable Levac's third point to be addressed: to relate the meaning of the findings to the study purpose and discuss the implications for future research (Levac et al., 2010). Finally, this approach will allow complete transparency at every stage of the process, so that any potential biases in the reporting are clear.

## Conclusion

This protocol outlines a method for systematically conducting a scoping review of peer-reviewed material, concerning the roles of vitamin D and UV exposure in MS onset, pathology and progression. The scoping review method enables disparate research to be collated and synthesised to provide insight into the different avenues of research which are all working towards further understanding the same outcome.

# Appendix C: Scoping review abstract data

| Authors/publication year | Study location | Sample | Study design | Outcome measures | Important results |
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| Midi et al., 2010 | Turkey | 42 RRMS; 24 controls | Case-control | 25(OH)D3 | 25(OH) D3 levels were below 25 nmol/L in 53%of MS and 17% of control patients. Vitamin D levels in MS patients were significantly lower than controls |
| Munger et al., 2010 | US | 222 MS, 444 controls | Prospective, nested case-control study | 25(OH)D, anti-EBNA antibodies | Increased anti-EBNA complex IgG antibodies and low 25(OH) D serum levels appear to be independent risk factors for MS |
| Hanwell et al., 2012 | Argentina, Canada, Finland, Italy, Russia, and the United States | 121 children with MS (all countries), 119 controls (Argentina, Canada, US) | Case-control | 25(OH)D; 25(OH)D2; ratio of 24,25(OH)D2 to 25(OH)D | Children with MS did not differ from controls in vitamin D status, prevalence of vitamin D insufficiency, nor the ratio of 24,25(OH)2D3:25(OH)D total. Children with MS were more likely to have detectable 25(OH)D2 levels than control participants |
| Golabchi and Shaygannejad, 2010 | Isfahan, Iran | 20 MS patients, 50 controls | Cross-sectional case-control study | 25(OH)D | Lower serum vitamin D levels were found in MS patients compared to the normal population, in spite of sufficient sun exposure in Isfahan region |
| Kumar et al., 2012 | India | 26 MS patients; 200 controls | Case-control | 25(OH)D; vitamin B12 | Serum vitamin D levels were low in the study population however B12 levels were within normal range. |
| Kucuk et al., 2015 | Turkey | 40 MS & 40 CIS, 60 controls | Case-control | 25(OH)D | 25(OH)D levels were significantly lower in patients than controls |
| Saraswathi et al., 2014 | India | 18 CDMS and 4 CIS; 17 controls | Case-control | Vitamin D | Serum 25(OH)D was significantly lower in patients with MS |
| Bilska et al., 2015 | Poland | 57 children with definite MS/CIS; 85 controls | Case-control | 25(OH)D | Cases and controls were vitamin D deficient, although more severe in cases. Vitamin D levels did not significantly correlate with disease incidence. FokI and BsmI polymorphism distribution did not differ between groups and there were no significant differences between vitamin D levels in the patients with individual polymorphisms |

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| Bilska and Kotulska-Jozwiak, 2012 | Poland | 29 children with clinically definite MS; 35 controls | Case-control | 25(OH)D | 86% of MS patients had 25(OH)D levels under 20 ng/ml. 14% of MS children had acceptable vitamin D status. 69% of controls had low vitamin D levels, 31% had sufficient vitamin D. Severe deficiency (< 10 ng/ml) was more common in MS children than controls. |
| Hanwell et al., 2009 | Canada | 25 children with MS | Prospective cohort study | 25(OH)D | 25(OH)D obtained at symptom onset differed significantly between children later diagnosed with MS and those remaining relapse-free. Each incremental 10 nmol/L rise in 25(OH)D was associated with a 20% reduction in the likelihood of MS diagnosis |
| Langer-Gould et al., 2015b | South California, USA | 457 incident cases of MS/CIS; 472 controls | Case-control | 25(OH)D | 25(OH)D levels were highest in whites followed by Hispanics and blacks. Higher 25(OH)D levels were associated with a lower risk of MS/CIS in whites particularly in those with levels greater than 75nmol/L, adjusted for obesity, smoking, IM and MS family history. There was no association in blacks or Hispanics |
| Martinelli et al., 2012 | Italy | 107 CIS patients | Single-centre retrospective study | 25(OH)D | There was a significant inverse correlation between 25(OH)D levels and risk of CDMS. The difference between serum 25(OH)D levels in patients who developed CDMS and patients who did not was significantly higher during summertime |
| Munger et al., 2014b | Finland | 197 MS cases, 400 controls | Nested case-control study | 25(OH)D | In utero 25(OH)D level was associated with a 41% decreased risk of MS among the offspring |
| Salzer et al., 2011 | Northern Sweden | 192 prospective MS and 37 from pregnancies where the child developed MS. 2 controls per MS case; 5 per MS pregnancy | Prospective case-control study; linkage | 25(OH)D | Prevalence of deficiency was very high. 4% of MS cases and 8% of controls had normal vitamin D levels ( >30 ng/ml) which were protective against MS. There was no decrease in MS risk with normal gestational vitamin D levels. Normal vitamin D levels in prospective blood samples were associated with a decreased risk of MS. Levels below 30 ng/ml did not further increase MS risk suggesting there is a threshold effect of vitamin D on MS risk. There was no effect of gestational vitamin D levels on MS risk |
| Langer-Gould et al., 2015a | USA | 89 blacks and 104 Hispanics with MS/CIS; 93 and 107 controls | Interim analysis of a case-control study | 25(OH)D | 25(OH)D levels are similar among black and Hispanic cases and controls. A 4ng/mL increase in serum 25(OH)D was not associated with a decreased risk in Hispanics; in blacks it is associated with a slight increase in MS risk after adjusting for history of IM, smoking and family history of MS |
| Xia, 2013 | China | 72 MS 24 NMO patients and 32 normal controls | Case-control | 25 (OH) D3 | Patients with MS or NMO had significantly lower levels of 25(OH)D3. There was no significant difference between MS patients and NMO patients. The 25(OH)D3 levels in SPMS patients were significantly lower than normal controls but were not significantly different from RRMS patients. 25 (OH) D3 levels were |

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| | | | | | significantly lower in RRMS patients during exacerbation compared with patients in remission |
| Runia et al., 2013 | Netherlands | 146 CIS patients | Prospective cohort study | 25(OH)D | Fatigue was associated with MS and conversion to CDM. When put into a model correcting for age, gender, MRI findings and 25(OH)D, fatigue was still significantly associated with MS but not with CDMS |
| Rose et al., 2012 | US | 500 MS patients | Cross-sectional? | Blood levels of vitamin D (no further information) | Females deficient in vitamin D (<20 ug/ml) had a significantly higher incidence in Contrasting Enhancing Lesions if they were DR2 serotype-positive. Males with low vitamin D (<30 ug/ml) were significantly worse in ambulatory status, disease progression, and brain atrophy than low vitamin D females |
| Simpson et al., 2009 | Southern Tasmania | 142 RRMS | Prospective cohort study | 25(OH)D | There was a statistically significant dose-dependent protective effect of 25(OH)D on relapse rates (9.6% reduction in relapse rates per 10nmol/L increase in serum 25(OH)D levels). For those with 25(OH)D levels at or above 40nmol/L, relapse rates were 33.6% lower relative to those with 25(OH)D below this level and for those at or above 80nmol/L, relapse rates were 56.8% lower relative to lower levels |
| Bove et al., 2014 | US | 173 MS patients | Retrospective longitudinal study | 25(OH)D | Higher EDSS was associated with lower 25(OH)D levels, lower testosterone levels, and higher adiposity markers |
| Muris et al., 2015a | Netherlands | 338 RRMS patients | Retrospective longitudinal study | 25(OH)D | Vitamin D status did not predict 3-year risk of conversion from RRMS to SPMS. However, in diagnostic blood samples of SPMS patients with a relatively short RRMS duration (n=19) 25(OH)D levels were significantly lower than in diagnostic samples of matched RRMS patients with no progression to SPMS |
| Niedziela et al., 2015 | Poland | 82 RRMS patients | Cross-sectional | Serum vitamin D | There were differences in PTH levels between deficient, insufficient, and normal vitamin D groups. Differences were also found in complaints about impaired mobility between the three groups. There were no differences between Vitamin D groups in EDSS |
| Jitprapaikulsan et al., 2014 | Thailand | 20 CIS, 34 MS and 76 NMO/NMOSDs patients | Cross-sectional | 25(OH)D | Vitamin D insufficiency is commonly found in Thai patients with CIS, MS and NMO/NMOSDs. There was no association between annualized relapse rate and vitamin D level |

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| Dastagir et al., 2013 | US | 100 RRMS or SPMS patients | Cross-sectional | Serum vitamin D | Mean 25(OH)D levels were 16.6 (6.8) in the African American MS and 23.7 (8.6) ng/ml in the European heritage MS patients. Vitamin D levels showed significant inverse correlation with EDSS and T2LL in both patient groups |
| Poellmann, W. L., Starck, M., Koehler, J. | Germany(?) | 161 MS patients | Prospective study | 25(OH)D3 | Women had higher mean vitamin D levels than men. Lower 25(OH)D3 levels were seen in younger patients than in patients 60-69 years. Small differences in mean vitamin D were seen in EDSS groups. High dose substitution with 20.000 IE led to a higher increase of 25(OH)D levels than weekly doses below 10.000 IE |
| Muris et al., 2015b | Netherlands? | 554 MS patients | Retrospective 3-year follow-up study | 25(OH)D | Baseline 25(OH)D status was associated with subsequent relapse risk, but only in the younger MS patients. Baseline 25(OH)D was not significantly associated with either disability or disability progression, irrespective of MS phenotype |
| Oliveira et al., 2012 | Brazil | 156 MS patients | Cross-sectional | 1,25(OH)2D; 25(OH)D | There was no significant correlation between disease progression and serum levels of 1,25(OH)D and/or 25(OH)D |
| Pakdaman et al., 2012 | Iran | 78 MS patients | Cross-sectional | 25(OH)D | There was a statistically significant inverse correlation between 25(OH) D3 concentration and EDSS score, observed in women only |
| Mowry et al., 2011 | USA | 469 MS patients | Five-year longitudinal MS cohort study | 25(OH)D3 | In multivariate analyses, each 10 ng/mL higher 25(OH)D3 level was associated with a 15% lower risk of developing a new T2 lesion and a 32% lower risk of a gadolinium-enhancing lesion. Higher vitamin D levels were insignificantly associated with a lower relapse risk |
| Bjornevik et al., 2012 | Norway and Italy | 733 cases and 1438 population based controls (Italy); 959 cases and 1718 population based controls (Norway) | Case-control | Standardized self-administered questionnaire | There was a significant inverse association between time spent outdoors and MS after adjustment for sex in Norway and Italy. In Norway the association was strongest with little sun exposure in the summer between age 16 and 18 years, while the period from age 0 to 5 years showed the strongest effect in Italy. There was a statistically significant inverse association in the winter in Italy but not in Norway. High sunscreen use between the age of 0 and 6 years was associated with an increased risk of MS in Norway after adjusting for sun exposure during the same period. |
| Fereidan-Esfahani and Etemadifar, 2014 | Iran | 97 paediatric MS cases and 97 controls | Case-control | Standardised questionnaire (phone interview or clinical surveillance) | Reported sunlight exposure 45 minutes per day or less before puberty compared with those who reported sunlight exposure greater than 2.5 hours per day before puberty had 17.66-fold increased odds of developing MS |

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| Steffensen et al., 2011 | North Norway | 68 RRMS patients who could walk at least 500m | Double-blind randomised controlled trial | 25(OH)D | At week 96, mean 25(OH)D was 123 nmol/L in the treatment group and 62 nmol/L in the placebo group. There was no definitive effect of vitamin D supplementation on disease activity or on results of functional tests |
| Alikhani and Kremenchutzky, 2009 | London, Canada | 15 RRMS patients | Open label pilot study, observational follow-up | Annualised relapse rate | 1 patient was diagnosed with Graves' disease, however no other serious metabolic conditions were diagnosed in this group. Effects on ARR and EDSS are inconclusive. |
| Zajicek et al., 2010 | UK and Ireland | Patients with CIS and two asymptomatic T2 lesions on MRI brain | Double blind randomised controlled trial | Clinical relapse and/or MRI evidence for new lesions as per McDonald criteria for MS | None reported |
| Ontaneda and Cohen, 2014 | USA | Sample size estimates using diffusion tensor imaging showed that a sample size of 20 subjects in each arm will have a power >90% to detect a 30% change in mean and longitudinal diffusivity. | 2 year randomized double blind placebo controlled study is proposed | 1) Diffusion tensor imaging of corticospinal tract and cortical thickness. 2) Variety of clinical, self-report, cognitive, and optical coherence tomography measures | None reported |
| Kimball et al., 2010 | Canada | | Prospective controlled trial | PBMC proliferation; T cell stimulation (TCS) scores | PBMC proliferation was reduced in treatment patients after one year and unchanged in controls. TCS dropped significantly in treatment patients, but not in controls. The proportion of patients with a TCS below the pre-determined positive threshold was greater in treatment patients. There were no differences between groups for other inflammatory markers |
| Fathi et al., 2015 | | 40 RRMS patients split into 2 groups | | | There was a statistically significant decrease in EDSS post UVR therapy, and a statistically significant increase in serum Vitamin D following UVR therapy. The number of new attacks post UVR therapy was decreased, although not significantly so |

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| Mattei et al., 2015 | | 3 patients (father and 2 sons) | Whole exome sequencing | | Variants affecting genes that interact with environmental risk factors: 11 variants affecting 10 genes that interact with EBV and 1 gene affected by deleterious and homozygous variants interacting with *VDR* that could alter the Vitamin D pathway. Also identified were 4 genes already related to MS by former GWAS studies |
| Briggs et al., 2013 | USA | 1,008 non-Hispanic White MS patients | Candidate gene study | | There were no significant associations between the vitamin D functional variants and any clinical phenotypes |
| Ebers and Ramagopalan, 2011 | Canada | 43 Canadian families with 4 or more with MS | Whole exome sequencing | | 43 individuals with MS (one/family) were sequenced, and over 58000 variants identified in each individual. A rare loss-of-function variant in the *CYP27B1* gene was identified. Further genotyping of other variants in over 11,000 individuals showed that rare *CYP27B1* variants conferred significant risk of MS |
| Hanwell et al., 2010 | Canada | 135 children (<16 years) with acute demyelination | Prospective | 25(OH)D | Hazard ratio for MS diagnosis in those carrying one or more DRB1*15 risk alleles was 2.58; the HR associated with each 10nmol/L increase in 25(OH)D was 0.87. In the 83 children with European ancestry, 23% were diagnosed with MS. For DRB1*15 carriers, the HR for MS diagnosis was 10.57; for each 10nmol/L increase in 25(OH)D, risk of diagnosis decreased by 29%. There was no significant interaction between DRB1*15 and 25(OH)D on MS risk |
| Nolan et al., 2012 | | 466 MS cases and 498 controls | Case-control | | *HLA-DRB1* promoter sequence variation may contribute to MS risk, however variation within the *VDRE* motif is not a dominant individual risk factor |
| Abraham et al., 2009 | | 551 unrelated MS patients | Candidate gene association study | Pre-validated questionnaire | There were no direct associations between MSSS and early-life UV exposure. The effect of explored polymorphisms on MSSS is retained only in patients with sunburn history, suggesting a complex interaction between skin type, UV exposure, and MC1R gene polymorphisms. |
| Runia et al., 2011 | | 493 MS patients; 2081 controls | Case-control study | 25(OH)D levels | *DBP* SNP rs7041 was associated with MS risk. Of the eight SNPs tested, six SNPs (in *CYP27B1*, *CYP24A1* and *DBP*, but not in *VDR*) had a significant association with serum 25(OH)D levels |
| Agliardi et al., 2010 | | 178 MS patients; 197 *HLA-DRB1*15*-positive controls, and 455 MS patients; 361 *HLADRB1*15*-negative controls | Case-control study | | The *VDR* rs731236 TT genotype in *HLA-DRB1*15*-positive individuals results in protection against MS, possibly because it is associated with an increased expression of *VDR* and/or *HLA-DRB1*15* |

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| Bettencourt et al., 2014 | Portugal | 426 patients; 261 controls | Case-control study | Serum 25(OH)D levels (available for only 154 MS patients) | Fok1 ff genotype frequency was significantly higher in the patient group compared to controls, however Taq1 showed no associations. Serum 25(OH)D levels revealed vitamin D deficiency or insufficiency in 66.9 % of the patients. There was also a negative correlation between vitamin D levels and disability (EDSS and MSSS) |
| Munger et al., 2014a | Multi-centre | 819 MS patients | Non-randomised, open-label, parallel-assignment clinical trial | 25(OH)D levels were measured at two to three time points during the trials | In GC, rs17467825 was significantly associated with reduced 25(OH)D levels by 10% comparing one to zero risk allele copies. In the DHCR7, rs4945008 was most significantly associated with a reduction of 25(OH)D by 7%. In *CYP2R1*, the most significant SNP was rs1993116 with a reduction of 25(OH)D levels by 6%. |
| Correale and Farez, 2012 | | 92 RRMS patients (58 in remission and 32 in exacerbations); 40 controls | Case-control | cis-UCA | Plasma levels of cis-UCA were significantly lower in MS patients compared to controls. No differences in plasma trans-UCA levels were found between groups, nor was any association found between disease activity and plasma cis-UCA levels |
| Lee et al., 2013 | China and Canada | 23 East Asian and 456 European heritage cases at the Vancouver clinic, and 47 cases at the Shanghai clinic. | Retrospective | Standardized retrospective questionnaire | Across all 3 cohorts, there was an upward trend of responses indicating "less than average" sunlight exposure later in life. There was a greater proportion of Shanghai cases reporting "more than average" sunlight exposure during both adult periods compared to East Asian cases in Vancouver |
| Tremlett et al., 2015 | US | In 2012/13, 151 incident MS cases and 235 controls | Case-control | Questionnaire | Women living in areas of high ambient UVB between ages 5-15 had a 48% lower risk of MS. Neither time spent outdoors in summer nor winter was associated with MS risk. However, in areas of low ambient UVB, less time spent outdoors in summer at ages 5-15 years saw a two-fold increased risk of MS |
| Lucas, 2012 | Australia | 282 first clinical diagnosis of CNS demyelination (FCD) cases; 558 controls | Case-control | Questionnaire, interview, physical examination, and biological sampling | There was an increased risk of being a FCD case in association with *HLA* class 1 and class II SNPs, and 3 SNPs associated with genes of the vitamin D pathway (24-hydroxylase; 1,25 hydroxylase; vitamin D receptor). FCD risk decreased with increasing 25(OH)D concentration and increased with a history of glandular fever and EBNA titre |

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| Olsson, 2013 | Sweden | 2200 incident MS cases and 4400 matched population-based controls | Case-control | | Smoking, oral tobacco use, lack of sun exposure/vitamin D, EBV infection, high EBNA1 fragment serology, obesity, and night shift work were associated with increased MS risk. Smoking interacted with *HLA-DRB1*15* and absence of *HLA-A*02*. There were similar interactions with EBV infection, and no interactions between sun exposure habits/vitamin D levels or night shift work |
| Disanto et al., 2011 | UK | 11,282 Scottish and 8,702 English MS patients | Cohort study | Serum vitamin D; ecological UV exposure based on residence | The distributions of MS births significantly differed from the general population in Scotland with April and May peaks and a September trough. MS risk inversely correlated with UV exposure during the second trimester of pregnancy in Scotland and England/Wales. Low vitamin D levels between the 5th and 8th months (in England/Wales) and in the third trimester in Scotland were also associated with MS risk |
| Baarnhielm et al., 2010 | Sweden | 1231 incident MS cases and 2682 controls | Case-control | Self-report UV exposure; 25(OH)D | Lowest UV exposure was associated with significantly increased risk of MS compared with highest exposure. Vitamin D was significantly lower in cases than controls. There was no interaction between *HLA-DRB1*15* and previous exposure to UV or vitamin D |
| Van Der Mei et al., 2014 | Australia | No info | Case-control | Genetic and environmental risk factors | Those with 2 to 5 risk factors had an increased odds of being a FCD case compared with no risk factors. Only *HLA-DR15* and history of IM interacted significantly on the additive scale. There were insignificant associations for an interaction between *HLA-DR15* and smoking, and *HLA-DR15* and low vitamin D. Five risk factors accounted for 64% of MS onset with *HLA-DR15*, ever-smoking and a measure of cumulative lifetime sun exposure contributing most |
| Leoni et al., 2014 | Italy | 684 cases, 1307 controls | Case-control | Vitamin D intake estimated per food portion and intake frequency | There was an inverse association between MS and fish consumption, especially anchovies, sardines, and mackerel (adjusted for sex, smoking, sun exposure, and vitamin D supplementation). Smoked meat and yoghurt consumption was also associated with a lower MS risk. Overall estimated mean intake of vitamin D was higher in controls vs cases |

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## Appendix D: Farming, foreign holidays, and vitamin D in Orkney

1) PLoS One paper

2) Supplementary Table 1: Principal components for socio-economic status variables

3) Supplementary Table 2: Missing data in the Orkney dataset in variables of interest

4) Supplementary Table 3: Comparison of people under 40 who holiday outside the UK at least once a year and people under 40 who holiday outside the UK less than once a year or never

PLOS ONE

RESEARCH ARTICLE

# Farming, Foreign Holidays, and Vitamin D in Orkney

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**Data Availability Statement:** The data contain individual-level phenotypic information from isolated human populations. The participants' signed consent and ethical permission do not allow that this information be publicly available, as the identity of specific individuals can be inferred from such data. Requests for the data should be made to the QTL-executive at the Institute of Genetics and Molecular Medicine (IGMM) by contacting James F Wilson (mailto:jim.wilson@ed.ac.uk).

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## Abstract

Orkney, north of mainland Scotland, has the world's highest prevalence of multiple sclerosis (MS); vitamin D deficiency, a marker of low UV exposure, is also common in Scotland. Strong associations have been identified between vitamin D deficiency and MS, and between UV exposure and MS independent of vitamin D, although causal relationships remain to be confirmed. We aimed to compare plasma 25-hydroxyvitamin D levels in Orkney and mainland Scotland, and establish the determinants of vitamin D status in Orkney. We compared mean vitamin D and prevalence of deficiency in cross-sectional study data from participants in the Orkney Complex Disease Study (ORCADES) and controls in the Scottish Colorectal Cancer Study (SOCCS). We used multivariable regression to identify factors associated with vitamin D levels in Orkney. Mean (standard deviation) vitamin D was significantly higher among ORCADES than SOCCS participants (35.3 (18.0) and 31.7 (21.2), respectively). Prevalence of severe vitamin D deficiency was lower in ORCADES than SOCCS participants (6.6% to 16.2% $p = 1.1 \times 10^{-15}$). Older age, farming occupations and foreign holidays were significantly associated with higher vitamin D in Orkney. Although mean vitamin D levels are higher in Orkney than mainland Scotland, this masks variation within the Orkney population which may influence MS risk.

## Introduction

Multiple sclerosis is a chronic, complex disease with genetic, environmental and behavioural factors implicated in its aetiology [1]. Greater distance from the equator is associated with increasing MS prevalence [2]; increasing latitude is also noted for weaker ultraviolet B (UVB) radiation which inhibits cutaneous production of vitamin D [3]. As such, one environmental risk factor is thought to be vitamin D deficiency, however, vitamin D is also a marker for exposure to UV radiation, the benefits of which may be independent of vitamin D production [4–6]. A variety of factors hinder or prevent UVB from reaching the earth's surface, including latitude and weather [7], or from initiating cutaneous vitamin D synthesis, such as sun protection creams and clothing cover.

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Although a recent systematic review of vitamin D status worldwide found that vitamin D concentrations do not appear to be dependent upon latitude [8], exposure to ultraviolet B (UVB) radiation from sunshine is the most potent source of vitamin D for humans [9]. In the United States, a latitudinal relationship exists between wintertime vitamin D and wintertime UV doses [10], likely resulting from the few days in which vitamin D can be produced at such latitudes [3, 10]. This latitudinal relationship further reflects the latitudinal MS prevalence distribution in the US [10]. Additionally, a significant relationship between regional UVB radiation and MS prevalence has been noted in France in the French farming population and their families [11]. Increasing MS prevalence was associated with decreasing ambient UVB; the trend was additionally found to be stronger in both wintertime and in women [11]. A similar relationship between decreasing UV and increasing prevalence has been identified in Newfoundland [12] and Australia [13].

Controversy remains regarding the role of vitamin D in chronic conditions; whilst deficiency may have a causal role in the aetiology of some diseases it may also simply be a biomarker for ill health. A body of evidence is however accumulating, suggesting a causal role for vitamin D in MS risk, pathology and progression [14–16]. A recent Mendelian randomisation study found genetically lowered 25-hydroxyvitamin D (25(OH)D) level by one standard deviation in log-transformed 25(OH)D was associated with a two-fold increased risk of MS [16]. Interactions between vitamin D and the major genetic risk factor, HLA-DRB1*1501, have been identified [17], and several rare variants conferring MS risk have been found in the *CYP27B1* gene, which encodes an enzyme which catalyses the conversion of 25(OH)D to the bioactive form [18]. Further, early exposure to vitamin D in-utero and in neonatal mice led to optimal numbers of invariant natural killer T cells [19], deficiency of which are observed in MS patients [19, 20]. Alongside the month-of-birth effect, where children born after a winter gestation are at higher risk of MS [21], the role of adequate in-utero vitamin D increasingly appears to be critical for future autoimmunity.

The beneficial role of UV exposure independent of vitamin D production has been supported in animal studies, using experimental autoimmune encephalomyelitis (EAE) as the model for MS. Continuous treatment with UVB was found to suppress clinical signs of EAE which, although leading to slight elevations in serum 25(OH)$D_3$, were insufficient to cause disease suppression by vitamin D [4]. Furthermore, suppression of EAE was found to occur upon irradiation of mice to narrow-band UV light, with a wavelength of between 300 and 315 nm and a peak of effectiveness at 311 nm. As vitamin D requires a wavelength between 270 and 300 nm, optimally between 295 and 300 nm to initiate cutaneous synthesis, the narrow band UV supressing EAE had no effect on 25(OH)D levels [5], strongly suggesting a role of UV exposure independent of vitamin D. In MS, an Australian multi-centre case-control study found higher sun exposure and higher vitamin D levels to be independently associated with lower risk for first demyelinating events [6].

Scotland, between latitudes 54° and 60° north, has inadequate strength of sunshine between October and March for vitamin D synthesis [22]; a cloudy climate year-round further leads to widespread vitamin D deficiency [23] strongly indicating limited UV exposure in the population. The protective effect of supplementation and sunny holidays on 25(OH)D in Aberdeen, a Scottish city at 57° north, has previously been noted in a study of postmenopausal women [24]. Orkney, an isolated archipelago ten to sixty miles from the north coast of Scotland, is an area of exceptionally high MS prevalence [25]. Seventeen of the 70 islands are inhabited with a predominantly rural population totalling 21,349 at the 2011 census. The 2011 census also revealed an ongoing agricultural tradition with 10% of the workforce employed in agriculture or fishing.

As an independent risk factor, or as a marker of UV exposure, it is important to understand the determinants of plasma vitamin D in the context of MS and other diseases of public health

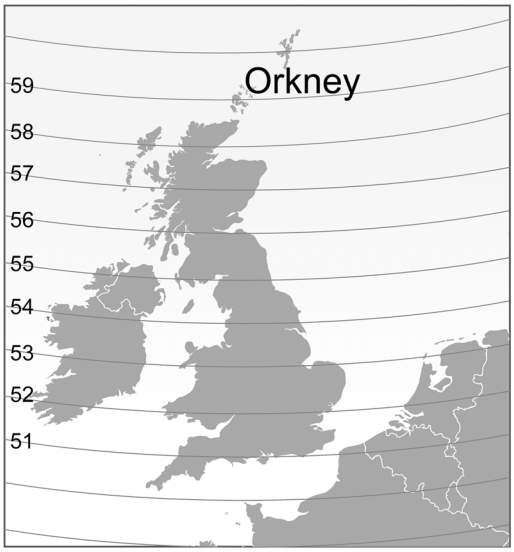


**Fig 1. Map of Orkney in relation to the Scottish mainland and north-west periphery of Europe, with 57th and 59th degrees of latitude.**

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importance. In this study we aimed to describe vitamin D levels in Orkney (Fig 1). This involved identifying the prevalence of vitamin D deficiency in Orkney compared to the Scottish mainland, and establishing the determinants of circulating plasma 25-hydroxyvitamin D (25 (OH)D) in Orkney.

## Methods and Materials

### Study Populations

The study population comprised Orkney Complex Disease Study (ORCADES) participants, recruited from 2005 to 2011, and Scottish Colorectal Cancer Study (SOCCS) controls, which ran from 1999 to 2006. Both these studies have been described in detail elsewhere [26, 27]. Briefly, ORCADES, a cross-sectional genetic epidemiology study concerned with identifying genetic factors that influence complex disease, comprised 2078 participants with at least one Orcadian grandparent. SOCCS, a case-control study of colorectal cancer in Scotland, included 2235 adult controls without colorectal cancer, identified from the Community Health Index in Scotland as being aged within 5 years their matched case, of the same sex and living in the same area. All participants provided written, informed consent prior to participation. Both studies have ethical approval from NHS Orkney, NHS Grampian or NHS Lothian.

### 25-hydroxyvitamin D measurement

Fasting blood samples were drawn from ORCADES participants using the Sarstedt Monovette system. Samples were processed and transferred for storage at -40$^0$ C, and later -80$^0$ C, until analysis. Blood was drawn from all SOCCS participants, processed and transferred for storage at -80$^0$ C. Both studies ran over multiple years, and therefore measures per month comprise blood drawn in multiple Januarys, multiple Februarys and so on.

Vitamin D status is determined by measuring circulating 25(OH)D which is generally considered the best indicator of vitamin D status [28]. Both 25(OH)$D_2$ and 25(OH)$D_3$ were measured using the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The total of the two measures was taken for total circulating 25(OH)D, however most samples from

both studies contained no 25(OH)$D_2$. The lower limit of detection using LC-MS/MS was 10 nmol/L. All samples were measured in the same laboratory following standard protocols; quality control procedures were performed according to current best evidence for 25(OH)D measurement in population studies [29].

A range of cut-offs to define sufficiency and deficiency have been proposed, however in line with other recent studies we considered circulating 25(OH)D of 50 nmol/L or over to be sufficient [30], 25 to 50 nmol/L to be at risk of deficiency or insufficient, and deficiency to be less than 25 nmol/L [31]. We additionally explored those at the lower end of deficiency, where we considered circulating 25(OH)D below 12.5 nmol/L to be severely deficient [32].

## Lifestyle factors

ORCADES participants attended clinics where several biometric measures were recorded. Each participant also completed a medical and lifestyle questionnaire from which vitamin D intake, physical activity (PA) and socioeconomic status (SES) were derived.

BMI was calculated as $kg/m^2$ and treated as a continuous variable. Height was taken without shoes and weight wearing only light clothing. Based on questionnaire data we derived a variable encompassing work and leisure time PA throughout the year. Participants classified leisure activity as either 1) light (mostly sitting, light housework) or 2) moderate exercise; and likewise work activity as 1) mostly sitting, 2) mostly standing, 3) manual work or 4) heavy manual work. Dietary vitamin D intake was estimated from two self-administered food frequency questionnaires, the cardiovascular disease questionnaire (CVDQ) and bone density questionnaire (BDQ). The CVDQ was treated as the primary source due to the higher response rate, however information contained in the BDQ that was not present in the CVDQ was merged to create the most comprehensive variable possible. Further, a research nurse-administered drug questionnaire sought information about medications and dietary supplements. Participants also described their frequency of taking holidays within or outside the UK (never, less than once a year, once a year, more than once a year). Although the Scottish Index for Multiple Deprivation (SIMD) is available for Orkney, the scattered and heterogeneous population means that concentrations of poverty or affluence are difficult to identify; moreover neighbouring islands are grouped together in units thus there is little discrimination [33]. Principal Components Analysis (PCA) is a statistical technique to reduce a number of variables into a few independent dimensions reflecting the underlying patterns in the data, and was used here to construct SES indices [34]. To establish a variable that differentiates between individuals, three SES variables were thereby derived from 10 questionnaire items with significant loadings in PCA (S1 Table). Additionally, we applied an occupational prestige score to questionnaire occupation information which was then included in the PCA [35]. Holidays, car age and council tax band loaded significantly onto the first component; housing tenure, length of car ownership and highest qualification loaded significantly onto the second component, and job prestige score, years in education and supervisory role at work loaded significantly onto the third. This third component captures "non-traditional" lifestyles reflecting managerial, administrative and professional positions in contrast to traditional agricultural work. Time outside in summer was summed from participants' estimates of average time spent without a roof covering on summer work and leisure days. Data from SOCCS included age, sex, month of blood sample and 25(OH)D measurement.

## Statistical analysis

We matched the ORCADES and SOCCS datasets on age to within two years (Table 1) to remove any differences arising from age structures. Matching was carried out blind excepting

**Table 1. Distribution of age and crude vitamin D in age-matched Orkney and mainland Scotland datasets.** The mainland dataset excludes people from above the 57th degree of latitude.

| | Orkney | Mean 25OHD | Mainland | Mean 25OHD |
|---|---|---|---|---|
| | No (%) | (nmol/L) | No (%) | (nmol/L) |
| Participants | 1453 | 36.2 | 1453 | 35.4 |
| < 40 | 46 (3.17) | 26.8 | 46 (3.17) | 36.5 |
| 40–49 | 263 (18.1) | 33.4 | 263 (18.1) | 39.5 |
| 50–59 | 399 (27.5) | 35.7 | 400 (27.5) | 38.7 |
| 60–69 | 466 (32.1) | 37.9 | 464 (31.9) | 33.3 |
| 70 + | 279 (19.2) | 39.5 | 280 (19.3) | 30.4 |
| Sex (male) | 590 (40.6) | 37.2 | 794 (54.6) | 35.4 |
| Sex (female) | 863 (59.4) | 35.6 | 659 (45.4) | 35.4 |

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dataset of origin and age. Because of the large effect of season on vitamin D levels, we standardised 25(OH)D measurements to the month of May; values obtained thereby represent those that would be expected if every sample were drawn in May [32]. The mean of monthly means in the mainland Scottish data is 34.4 nmol/L; in the Orcadian data the mean of monthly means is 37.7 nmol/L. The May means are 33.8 nmol/L and 35.5 nmol/L, respectively. We used May-standardised measurements for all analyses concerned with determinants of vitamin D, and also to compare Orkney and mainland Scotland in deficiency levels. For analyses concerned with vitamin D and time of year we used crude 25(OH)D measures. Data are presented as mean (standard deviation).

We plotted crude 25(OH)D by location as a density plot and by month, and compared using a t-test. We compared vitamin D by age group using t-tests. To compare levels of deficiency in Orkney and mainland Scotland, we divided participants into groups of deficiency and plotted May-adjusted vitamin D for each deficiency group and location and tested for differences using chi-square tests.

For determinants of May-adjusted 25(OH)D in Orkney, we ran a series of bivariable models of May-adjusted 25(OH)D against environmental and demographic variables of interest. Those that were significant were put into a multivariable model. These significant variables comprised BMI, age at venepuncture, foreign holidays, PA, SES, dietary vitamin D and working status. Sex was also a covariate. A large percentage of missing data (S2 Table) was imputed using Multiple Imputation of Chained Equations (MICE)[36] after excluding 28 individuals with missing outcome data. We ran 68 cycles of 100 imputations and pooled the results in a linear regression model. We ran the same model using complete cases only. Statistical tests were two-sided with $p<0.05$ taken as significant. Finally, we applied a one-way ANOVA to compare mean May-adjusted 25(OH)D across the three groups of participants that we identified as a result of our analyses.

We assessed homoscedasticity by inspection of a QQ plot, and distribution of residuals using a histogram with superimposed normal curve. Independence was checked using the Durbin Watson statistic, multicollinearity and outliers using the vif statistic and Cook's distance, respectively. All analyses were conducted using R software version 3.2.0 [37].

## Results

For this study, 64 individuals were excluded from ORCADES who were not resident in Orkney, 10 who had MS, as well as 8 duplicate measures. Characteristics of ORCADES participants are presented in Table 2. Twenty-three people were excluded from the Scottish Colorectal Cancer

**Table 2. Characteristics of ORCADES Study participants (n = 1972).**

| Characteristic | No or mean | SD | % or range |
|---|---|---|---|
| Age at venepuncture (years) | 53.4 | 15.3 | 16.5–100.2 |
| Sex | | | |
| Female | 1191 | - | 60.4 |
| Male | 781 | - | 39.6 |
| Body Mass Index (kg/m$^2$) | 27.7 | 4.9 | 16.9–53.9 |
| Vitamin D intake (μg) | 4.4 | 3.1 | 0.00–34.1 |
| Physical activity* | 5.1 | 1.2 | 3.0–8.0 |
| Summer minutes outdoors | 223 | 142 | 4.8–900 |
| Working | 1367 | - | 69.3 |
| Retired | 547 | - | 27.7 |
| Holidays outside the UK | | | |
| < once a year | 1472 | - | 74.6 |
| Once a year | 329 | - | 16.7 |
| > once a year | 105 | - | 5.3 |
| Years in education | 16 | 1.2 | 1.0–23 |
| Qualification level | | | |
| O & standard grades, CSE | 275 | - | 13.9 |
| Highers, A levels | 787 | - | 39.9 |
| Certificates/diplomas | 739 | - | 37.5 |
| Bachelor/Master degree | 88 | - | 4.5 |
| Doctorate | 13 | - | 0.7 |

* Physical activity scored from 1 (mostly sitting; inactive) to 4 (heavy manual labour; active) across different domains within work and leisure. Each score is the sum of answers creating an individual value for each participant.

doi:10.1371/journal.pone.0155633.t002

Study who lived above the 57$^{th}$ degree of latitude. For comparison analyses, data were age-matched giving a final count of 1453 people in each dataset.

Using age-matched data we compared mean 25(OH)D in Orkney to mainland Scotland (Fig 2). Orkney had significantly higher crude 25(OH)D than mainland Scotland (Orkney 35.3 (18.01), Mainland 31.7 (21.18), $t(2800) = -4.93$, $p = 8.5 \times 10^{-7}$). Mean 25(OH)D was higher in Orkney for every month except August when the mainland peaks at ~50 nmol/L. The distribution of vitamin D levels is shifted to the right in Orkney (Fig 3). We compared vitamin D in Orkney and mainland Scotland by age group (Table 3). Results for each age group are significantly different, however in the under 40s, 40 to 49 and 50 to 59 age groups, mainland Scotland has higher vitamin D, whilst in the 60 to 69 and over 70s age groups, Orkney has significantly higher vitamin D. Comparing Orkney with the mainland by deficiency group, we found that more people in Orkney had insufficient vitamin D, $\chi^2$ (1, N = 2863) = 30.3, $p = 3.8 \times 10^{-8}$; however, Orkney had significantly fewer people with circulating 25(OH)D of <12.5nmol/L (severely deficient) compared with the mainland, $\chi^2$ (1, N = 2863) = 64.3, $p = 1.1 \times 10^{-15}$ (Fig 4).

To explore correlates of vitamin D in Orkney we ran two multivariable regression analyses, using both imputed data and complete cases. Each model yielded similar results (Table 4). Variables significantly associated with higher 25(OH)D included lower BMI, more foreign holidays, older age and increased PA. Associated with lower 25(OH)D was the "non-traditional" SES grouping.

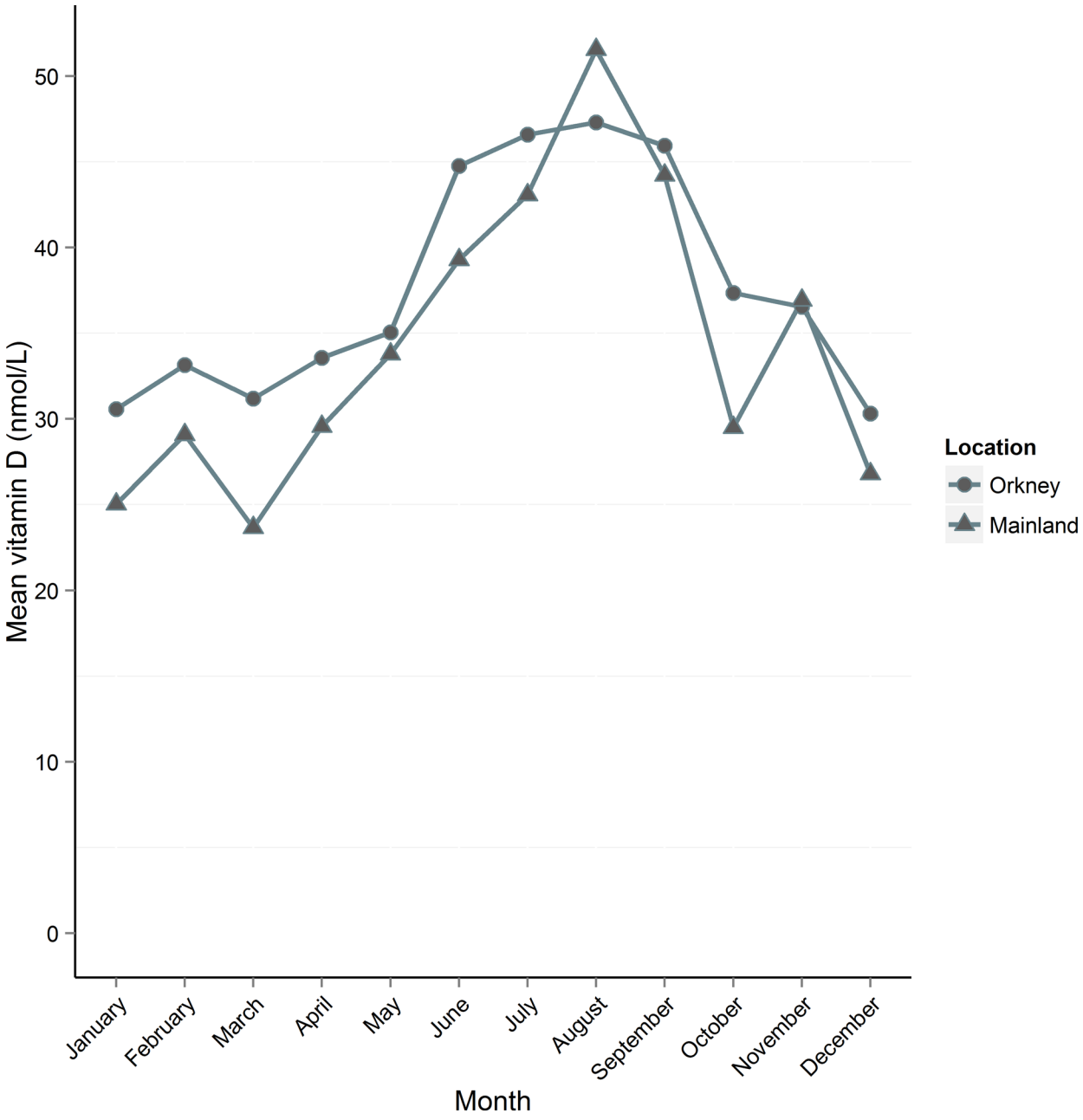


**Fig 2. Mean crude vitamin D concentration (nmol/L) per month by location, using age-matched data.** Orkney's mean vitamin D is higher than the mainland for every month except August and November. Each study ran over consecutive years and measurements taken in the same month each year were pooled.

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The association between older age and higher vitamin D required further exploration; we began by comparing foreign holiday-takers and their non-holidaying counterparts. We found that people over 50 were significantly more likely to take foreign holidays at least once a year compared to people under 50 (Table 5, Fig 5) ($\chi^2(1) = 6.4$, $p = 0.0083$). We termed this the 'Saga' effect. Additionally, we found that foreign holidays had a stronger effect on people over 50 who had their blood drawn in the low vitamin D (weaker UVB) season (October to March)

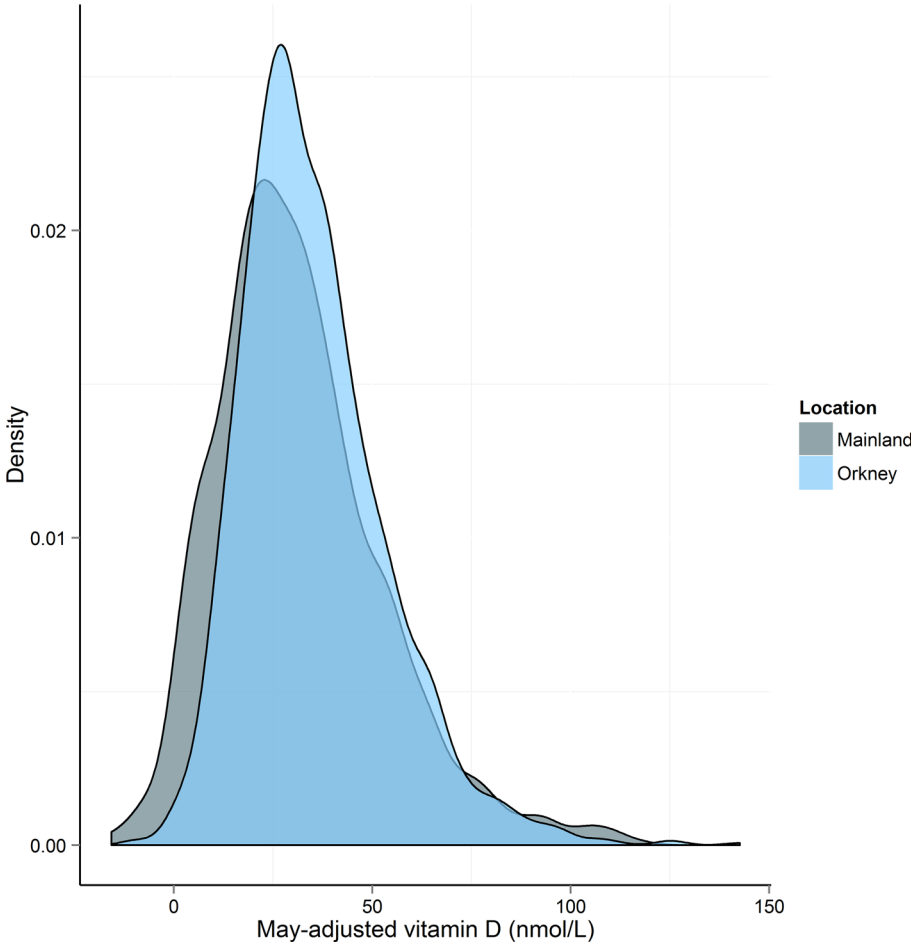


**Fig 3. Comparison of May-adjusted vitamin D distribution in Orkney and mainland Scotland using age-matched data.** The distribution for Orkney is to the right of the distribution for the mainland, reflecting the lower prevalence of severe deficiency, and peaks higher.

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compared with people who had their blood drawn in the high vitamin D (stronger UVB) season (April to September) (Table 6).

To explore under-40s further, we ran the same analyses comparing those who do and do not holiday outside the UK that were used for over-50s (S3 Table). The same results were significant, excepting body fat percentage, socio-economic status 2, age, and summer minutes spent outside.

**Table 3. Comparison of vitamin D in Orkney and mainland Scotland by age group.**

| | N = Orkney; Scotland | Orkney mean crude 25(OH)D | Mainland mean crude 25(OH)D | t-test | p-value |
|---|---|---|---|---|---|
| < 40 | 46; 46 | 26.8 | 36.5 | -2.67 | 0.009 |
| 40–49 | 263; 263 | 33.4 | 39.5 | -3.13 | 0.002 |
| 50–59 | 399; 400 | 35.7 | 38.7 | -1.97 | 0.049 |
| 60–69 | 466; 464 | 37.95 | 33.28 | 3.59 | 0.0004 |
| 70+ | 279; 280 | 38.51 | 30.39 | 4.84 | $1.6 \times 10^{-6}$ |

doi:10.1371/journal.pone.0155633.t003

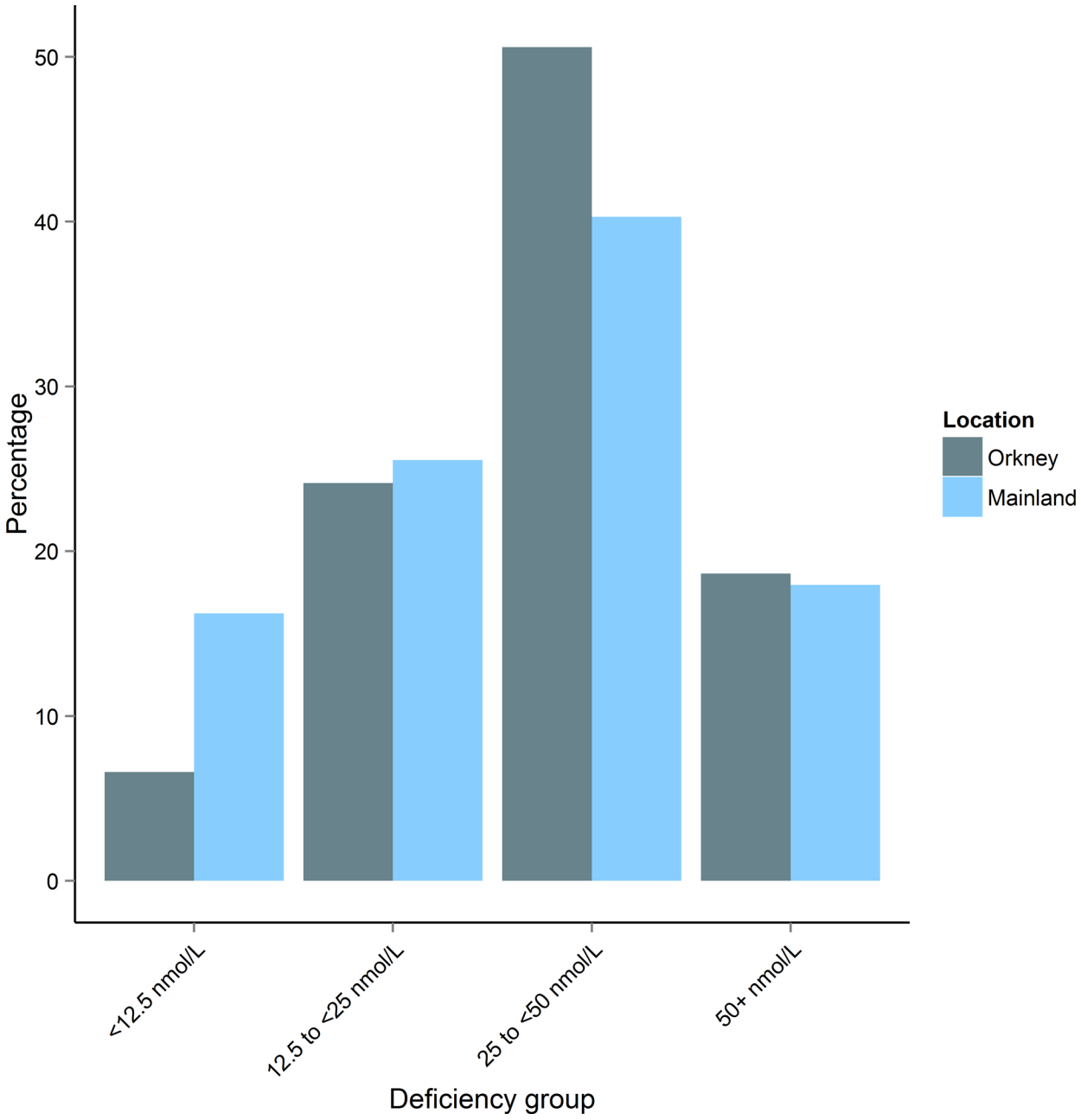


**Fig 4. Comparison of percentage of people in May-adjusted vitamin D deficiency groups by location.** The main differences occur in the severely deficient group (<12.5 nmol/L) which has significantly fewer people from the Orkney sample ($\chi^2(1) = 64.2$, $p = 1.10 \times 10^{-15}$), and the 'at risk' category (25-<50 nmol/L) which has significantly fewer people from the mainland Scottish sample ($\chi^2(1) = 30.3$, $p = 3.78 \times 10^{-8}$).

doi:10.1371/journal.pone.0155633.g004

We also identified a 'farmer effect' (Table 7). Participants employed in "traditional" agricultural occupations that kept them outdoors had significantly higher mean vitamin D levels than participants in non-traditional professions that kept them indoors (farmers 36.9 (18.0), non-farmers 33.8 (11.8), $t(383) = 2.46$, p = 0.014). Further, farmers tended to be older.

To test for differences in mean vitamin D across the 'Saga' group, farmers, and non-farmers who are under 50 and do not take foreign holidays, we did a one-way ANOVA. This ANOVA

**Table 4. Results of linear regression for complete cases and imputed data using May-adjusted vitamin D as the outcome.**

| | Multivariable models | | | |
|---|---|---|---|---|
| | Model 1[a] (n = 628) | | Model 2[b] (n = 1949) | |
| **Predictors** | **Est (95% CI)** | **p-value** | **Est (95% CI)** | **p-value** |
| Intercept | 32.4 (18.5, 46.2) | $5.25x10^{-6}$ | 30.3 (22.2, 38.3) | $2.2x10^{-13}$ |
| Body mass index (kg/m$^2$) | -0.75 (-1.02, -0.47) | $1.95x10^{-7}$ | -0.54 (-0.70, -0.38) | $7.5x10^{-11}$ |
| Holidays outside the UK | | | | |
| < once a year | -0.93 (-4.38, 2.52) | 0.59 | 0.75 (-1.34, 2.85) | 0.48 |
| Once year | 5.03 (0.20, 9.86) | 0.041 | 6.47 (3.47, 9.47) | 0.000024 |
| > once a year | 18.7 (11.3, 26.2) | $1.04x10^{-6}$ | 13.5 (9.07, 18.0) | $3.4x10^{-9}$ |
| Age at venepuncture | 0.24 (0.11, 0.36) | 0.0003 | 0.14 (0.07, 0.22) | 0.00030 |
| Physical activity | 1.66 (0.58, 2.75) | 0.003 | 1.42 (0.65, 2.19) | 0.00032 |
| Socio-economic status 3 ("non-traditional") | -2.10 (-3.57, -0.64) | 0.005 | -1.74 (-2.71, -0.78) | 0.00043 |
| Summer minutes outside | 0.0046 (-0.00398, 0.013) | 0.29 | 0.006 (-0.00028, 0.012) | 0.062 |
| Socio-economic status 2 | 0.32 (-1.30, 1.94) | 0.70 | 0.69 (-0.34, 1.72) | 0.19 |
| Vitamin D intake (μg) | 0.201 (-0.18, 0.60) | 0.30 | 0.14 (-0.17, 0.46) | 0.37 |
| Working (not retired) | -2.07 (-6.61, 2.47) | 0.37 | -0.18 (-2.45, 2.09) | 0.88 |
| Sex (male) | 1.06 (-1.55, 3.67) | 0.43 | -0.12 (-1.77, 1.52) | 0.88 |
| Socio-economic status 1 | -0.013 (-1.85, 1.82) | 0.99 | -0.075 (-1.17, 1.02) | 0.89 |

[a] Model 1 constructed using complete cases in the original dataset, $R^2 = 0.204$.

[b] Model 2 constructed using 68 datasets with missing data completed by imputation (100 cycles), $R^2 = 0.111$ (People missing outcome data were excluded from the imputation model).

Socio-economic status was derived from principal components analysis.

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was significant: Welch's $F(2, 481.99) = 54.49$, $p = 2.2 \times 10^{-16}$, and we therefore concluded that vitamin D varies significantly across these groups with people over 50 who take foreign holidays having higher vitamin D than farmers, who had higher vitamin D than non-farmers and people under 50 who remain in the UK (Fig 6).

## Discussion

We aimed to compare vitamin D levels in Orkney and mainland Scotland, and to identify the determinants of Orkney vitamin D. Definitions of vitamin D deficiency are much discussed, however it has been proposed that circulating 25(OH)D above 50 nmol/L are sufficient [30, 38]. Vitamin D status in Scotland has been previously explored [32]. Deficient and high risk individuals comprised 63.4% in the previous study; in our Orkney dataset deficient and high risk individuals comprised 65.3%. However, people with severe deficiency (<12.5 nmol/L) comprised 11.8% of the former study and only 5.0% of the latter. Therefore although perhaps initially surprising that mean vitamin D was higher in Orkney despite the higher latitude, the smaller percentage of people with severe deficiency in Orkney led to this elevation. In both datasets the majority are either deficient or at risk of deficiency which could have significant health implications [9].

Ability to synthesise vitamin D decreases with age [39]; it is well established that older age is associated with lower vitamin D and increased deficiency risk [32, 40]. However, we found that lifestyle factors particular to Orkney contributed to better vitamin D in older compared to younger people.

**Table 5. Comparison of people over 50 who holiday outside the UK at least once a year (n = 281) and people over 50 who holiday outside the UK less than once a year or never (n = 851).** Unpaired t-tests applied to continuous data; chi-square tests applied to categorical data.

| | Over-50s, holiday | Over-50s, no holiday | t-test or | p-value |
|---|---|---|---|---|
| | No (%) or Mean (SD) | No (%) or Mean (SD) | Chi-square | |
| Socio-economic status 1 | 1.01 (0.76) | -0.30 (0.92) | -22.6 | $<2.2x10^{-16}$ |
| Job prestige score | 0.32 (1.08) | -0.19 (0.96) | -7.09 | $5.1x10^{-12}$ |
| Socio-economic status 3 ("non-traditional") | 0.18 (1.05) | -0.29 (0.92) | -6.50 | $2.1x10^{-10}$ |
| Supervisory role at work | | | | |
| Yes | 186 (66.4) | 377 (45.1) | | |
| No | 94 (33.6) | 459 (54.9) | 38.2 | $6.4x10^{-10}$ |
| Years in education | 15.9 (1.26) | 15.5 (1.14) | -5.39 | $1.1x10^{-6}$ |
| Body mass index ($kg/m^2$) | 27.6 (3.91) | 28.9 (5.03) | 4.41 | $1.2x10^{-5}$ |
| Highest qualification | | | | |
| O & standard grades, CSE* | 33 (11.8) | 102 (12.1) | | |
| Highers, A levels** | 114 (40.7) | 246 (29.3) | | |
| Certificates/diplomas | 113 (40.4) | 461 (54.8) | | |
| Bachelor/Master/PhD | 20 (7.14) | 32 (3.81) | 22.2 | $5.8x10^{-5}$ |
| Summer minutes | 252 (146) | 214 (136) | -3.38 | 0.001 |
| Age | 62.9 (7.62) | 64.4 (8.63) | 2.55 | 0.01 |
| Bodyfat % | 33.7 (7.51) | 34.9 (8.07) | 2.34 | 0.02 |
| Socio-economic status 2 | 0.43 (0.76) | 0.32 (0.76) | -2.06 | 0.04 |
| Vitamin D intake (μg) | 5.09 (3.60) | 4.78 (3.13) | -1.12 | 0.26 |
| Physical activity | 5.21 (1.20) | 5.16 (1.26) | -0.37 | 0.71 |

* School examinations taken in the UK ~16 years of age

** School examinations taken in the UK ~18 years of age

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Participants who took foreign holidays had higher vitamin D than those who did not take foreign holidays; furthermore, taking foreign holidays increased by age group. Less than 20% of under-40s in our Orkney sample took foreign holidays, whereas over 30% in the 70 and over group reported leaving the UK at least once a year. Weak sunshine within the UK leads to fewer opportunities for UV exposure and UVB-mediated vitamin D synthesis, and the effect of foreign holiday sun exposure has been previously associated with improved vitamin D in Scotland [24]. Orkney mean vitamin D remained higher than mainland Scotland throughout the year with the exception of two months: August and November. Although we were unable to explore this further, mainland Scotland's August elevation of vitamin D could be attributed to the effect of holidays, following one month after school holidays. In November, however, mainland Scotland's mean vitamin D levels were only minimally higher than Orkney. We found that mean vitamin D in people 50 and over taking foreign holidays was significantly higher than vitamin D levels in the rest of the sample. Foreign holidays contributed more to vitamin D levels on blood drawn in months of weaker UVB. We were unable to explore at what time of year people take holidays, however this finding suggests that foreign holidays become more important as a source of UV exposure and therefore vitamin D for Orkney residents in winter. As older people tend to have more freedom to travel outside peak season, they are able to derive most benefit by seeking sun in seasons of scarce sunshine in Orkney. People over 50 who take foreign holidays were found to differ from those who do not take holidays mainly in variables denoting financial security. They were also more likely to have lower BMI and body fat percentage suggesting possible healthier lifestyles than their non-holidaying counterparts.

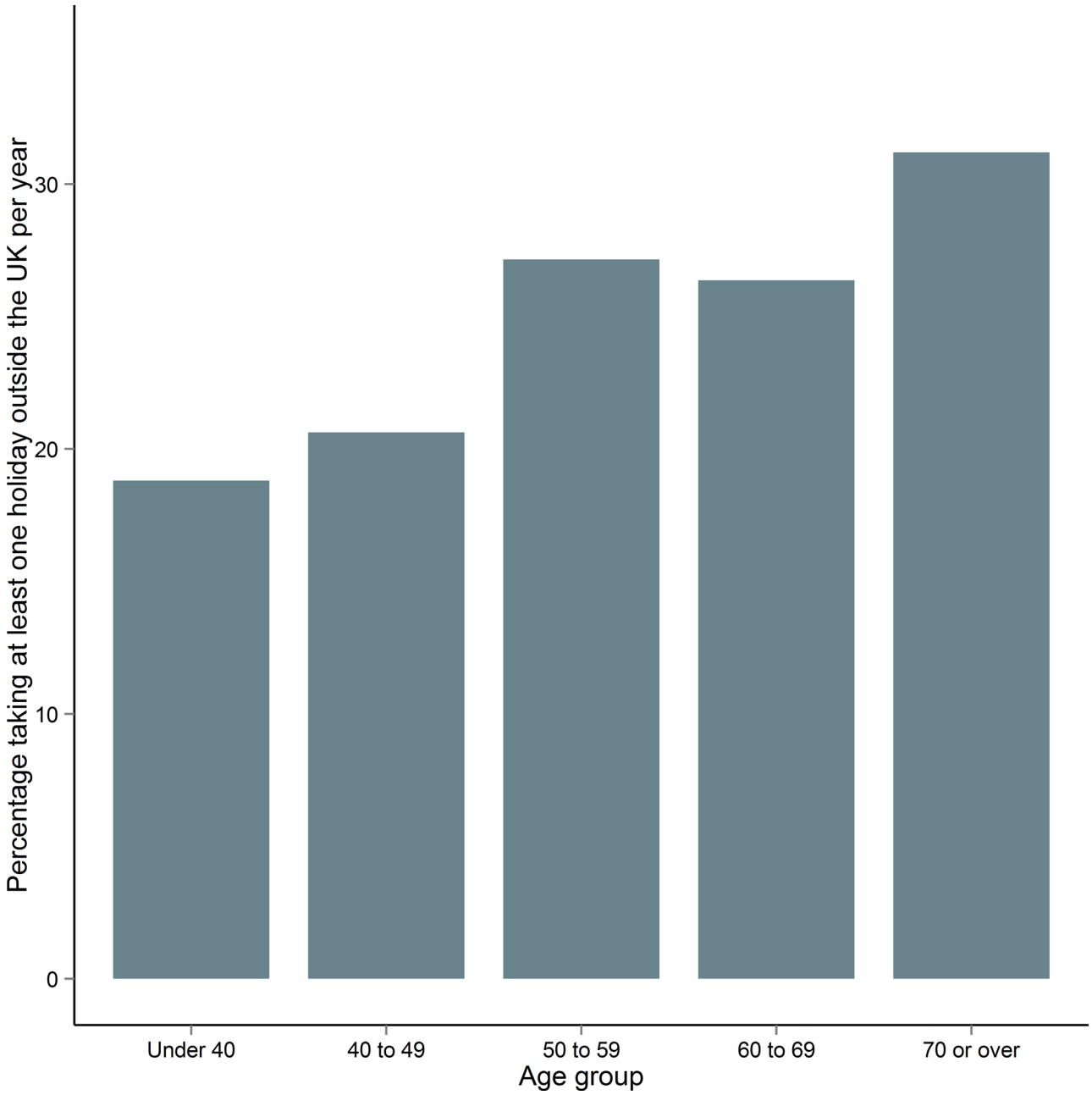


**Fig 5. Percentage of people per age group in ORCADES who holiday outside the UK at least once a year.** People over 50 take significantly more holidays than those under 50.

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**Table 6. Mean May-adjusted vitamin D (nmol/L) according to season of venepuncture (high season (April-September, n = 96) vs low season (October-March, n = 185)) in people over 50 who take a holiday outside the UK at least once a year.** Linear regression with May-adjusted vitamin D as the outcome.

| | Std Beta | Beta (95% CI) | Std error | p-value |
|---|---|---|---|---|
| High season over-50s | 0.17 | 7.86 (3.57–12.2) | 2.18 | 0.00035 |
| Low season over-50s | 0.19 | 8.33 (5.23–11.4) | 1.57 | $1.75 \times 10^{-7}$ |

doi:10.1371/journal.pone.0155633.t006

**Table 7. Comparison of farmers (n = 265) and non-farmers (n = 1649) on variables of interest in Orkney.** Farmers are anyone who identified their primary profession as farmer. Unpaired t-tests applied to continuous data; chi-square tests applied to categorical data.

| | Farmers | Non-farmers | t-test or | p-value |
|---|---|---|---|---|
| | No (%) or Mean (SD) | No (%) or Mean (SD) | Chi-square | |
| Age | 60.7 (11.4) | 52.4 (11.5) | -8.86 | $<2.2x10^{-16}$ |
| Socio-economic status 3 ("non-traditional") | -0.30 (0.58) | 0.10 (1.02) | 8.77 | $<2.2x10^{-16}$ |
| Years in education | 15.4 (1.01) | 16.1 (1.24) | 10.16 | $<2.2x10^{-16}$ |
| Socio-economic status 1 | -0.44 (1.00) | 0.09 (0.97) | 7.86 | $5.4x10^{-14}$ |
| Physical activity | 5.87 (1.23) | 5.10 (1.21) | -7.60 | $8.9x10^{-13}$ |
| Highest qualification | | | | |
| O & standard grades, CSE* | 24 (9.2) | 251 (15.3) | | |
| Highers, A levels** | 84 (32.1) | 702 (42.8) | | |
| Certificates/diplomas | 153 (58.4) | 586 (35.8) | | |
| Bachelor/Master/PhD | 1 (0.4) | 100 (6.1) | 55.9 | $4.3x10^{-12}$ |
| Bodyfat % | 30.4 (8.7) | 33.1 (8.6) | 4.51 | $9.1x10^{-6}$ |
| Supervisory role at work | | | | |
| Yes | 109 (41.3) | 866 (53.2) | | |
| No | 155 (58.7) | 763 (46.8) | 12.8 | 0.0003 |
| Socio-economic status 2 | 0.28 (0.92) | 0.07 (0.92) | -3.39 | 0.0008 |
| Body mass index ($kg/m^2$) | 28.4 (4.80) | 27.7 (4.96) | -2.12 | 0.04 |
| Vitamin D intake (μg) | 4.71 (2.93) | 4.40 (3.20) | -1.29 | 0.19 |
| Summer minutes | 236 (151) | 221 (141) | -1.21 | 0.23 |

* School examinations taken in the UK at ~16 years of age

** School examinations taken in the UK at ~18 years of age

doi:10.1371/journal.pone.0155633.t007

We also examined under 40s, the age group in which MS is most likely to be diagnosed and pregnancies are most likely to occur, thereby potentially conferring risk to the unborn child. In this group, we found that the main differences in those who do or do not take holidays out of the UK are related to financial security. Only 75 of the 400 people who are under 40 reported leaving the UK for a holiday at least once a year, therefore, in the most at-risk group, inadequate UV exposure in Orkney is compounded by a low prevalence of foreign holidays.

The 'non-traditional' SES group derived from PCA, comprising job prestige score, education years and supervisory role at work, was associated with vitamin D. These variables, reflecting "non-traditional" lifestyles of managerial, administrative and professional occupations in contrast to traditional agricultural work, related to farmers and non-farmers. Farmers were found to be slightly less educated, possibly as a result of leaving school at the minimum leaving age about half a year to a year before the first set of examinations. Farmers were also less likely to describe themselves as having a supervisory role at work than non-farmers, and also had a slightly lower-than-average job prestige score. The inverse association between vitamin D and a higher score in this variable means that farmers, who scored lower, had better vitamin D. Farmers in our cohort were also more likely to be older than non-farmers, further contributing to our finding of vitamin D increasing with age.

Physical inactivity and obesity have previously been related to low vitamin D in a large American cohort [41]; the association between lower BMI and higher 25(OH)D is also well established [42]. The mechanism for lower vitamin D in the presence of higher BMI is thought to be a result of increased deposition of vitamin D in body fat [43], making BMI a proxy for adiposity. However, BMI does not distinguish between body fat and fat free mass, and is not

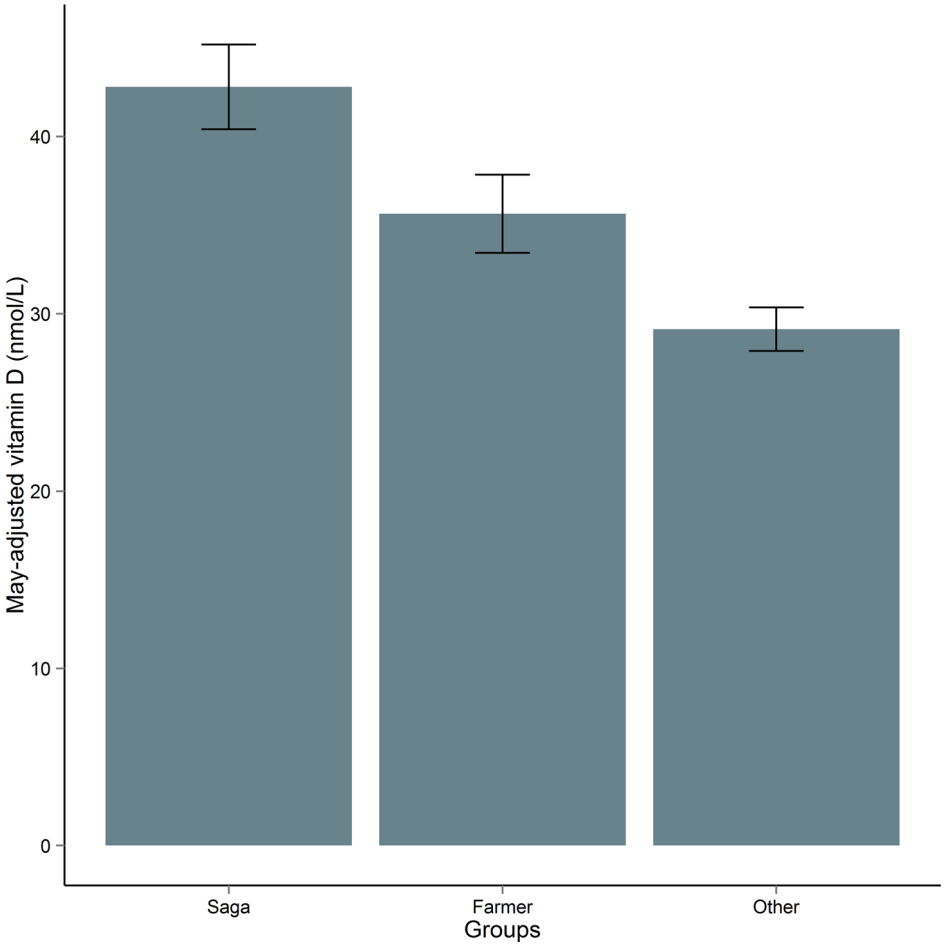


**Fig 6. Mean May-adjusted vitamin D (nmol/L) in different groups in ORCADES.** 95% confidence interval bars are given. "Saga" refers to people over 50 who take a holiday outside the UK at least once a year; "Farmer" to people who identified their primary profession as farming; "Other" is people under 50 who take a holiday outside the UK less than once a year, and are not farmers.

doi:10.1371/journal.pone.0155633.g006

always a reliable indicator of adiposity in people with lower body fat but greater muscle mass [44]. The farmers in our cohort reflected this difficulty: they had lower body fat percentage but higher BMI than non-farmers. We found farmers were more active and leaner than non-farmers and it is perhaps therefore fair to assume they have higher than average muscle mass. Nevertheless, in the multivariable models BMI followed what is expected.

Farmers had mean vitamin D significantly higher than non-farmers, but significantly lower than people over 50 who take foreign holidays. Summer minutes outside was not significant in the multivariable analyses, however we performed a t-test for farmers versus non farmers and time spent outside. Farmers, perhaps unsurprisingly, were found to spend significantly more time outside than non-farmers which enables maximisation of even the smallest window of vitamin-D strength sunshine. Although Zgaga et al. found higher vitamin D consumption led to slight improvements in plasma vitamin D [32], we found that diet was not associated with vitamin D in Orkney. However, difficulties involved in building a variable with the available data likely contributed to this finding.

Both studies were recruited on an 'opt in' basis which may result in the samples representing a healthier than average population; however a strength of this study was the large number of

participants in each cohort. Particularly novel was the number of farmers in our Orkney cohort, enabling us to explore vitamin D in a select group within a rural population which is not often studied. All vitamin D samples from both cohorts were analysed in the same laboratory using the same procedures, helping maintain consistency and reliability of results. We had access to a variety of detailed measures to explore vitamin D in Orkney; however data on time spent outside and vitamin D intake were somewhat limited and these may thus be more strongly implicated in vitamin D than we were able to detect. Nevertheless, we found significant effects and reliable relationships for vitamin D with foreign holidays, BMI, physical activity and age.

## Conclusion

Mean vitamin D in Orkney was higher than mainland Scotland, driven largely by a lower percentage of individuals with severe deficiency in Orkney. Overall concentrations in both cohorts were low with most people either deficient or at risk of deficiency, suggesting that UV exposure for much of the year is low. Older Orkney residents were more likely to have better vitamin D than younger residents, largely resulting from the 'Saga' and 'Farmer' effects. Those most at risk of deficiency in Orkney were under 40, an age group traditionally considered at lower risk of deficiency, but at increased risk of MS diagnosis. Within these main child-bearing years, a lack of UV exposure and vitamin D deficiency may result in significant autoimmune implications for offspring. The significant contribution of foreign holidays to Orkney vitamin D is consistent with the findings of previous UK studies; the importance of foreign holidays in providing adequate UV exposure to UK residents is underappreciated. We have found that younger ages are more at risk from inadequate UV exposure and vitamin D deficiency in Orkney, a county with a very high prevalence of MS. Further research exploring the relationship between vitamin D and quantitatively-measured exposure to UV radiation from sunshine and physical activity, as well as more detailed dietary information in Shetland, the most northerly UK county with an MS prevalence lower than Orkney, would help further elucidate the roles of UV exposure and vitamin D as MS risk factors in these islands.

## Supporting Information

**S1 Table. Principal components for socio-economic status variables.** The most significant loadings are in bold.
(DOCX)

**S2 Table. Missing data in the Orkney dataset in variables of interest**
(DOCX)

**S3 Table. Comparison of people under 40 who holiday outside the UK at least once a year (n = 75) and people under 40 who holiday outside the UK less than once a year or never (n = 325).** Unpaired t-tests applied to continuous data; chi-square tests applied to categorical data.
(DOCX)

## Acknowledgments

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## Author Contributions

Conceived and designed the experiments: EW JFW RM. Performed the experiments: EW. Analyzed the data: EW LZ SR. Contributed reagents/materials/analysis tools: HC MGD JFW. Wrote the paper: EW LZ SR SW HC MGD RM JFW.

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**Supplementary table 1.** Principal components for socio-economic status variables. The most significant loadings are in bold.

| | PC1 | PC3 | PC2 |
|---|---|---|---|
| Holidays in the UK | **0.70** | 0.17 | 0.01 |
| Holidays outside the UK | **0.65** | 0.28 | 0.16 |
| Car age | **-0.55** | 0.14 | -0.45 |
| Council tax band | **0.45** | 0.24 | -0.25 |
| Boat ownership | 0.33 | 0.05 | -0.02 |
| Job prestige score | 0.16 | **0.76** | 0.21 |
| Years in education | 0.16 | **0.72** | -0.19 |
| Supervisory role at work | 0.16 | **0.52** | 0.16 |
| Housing tenure | 0.08 | 0.07 | **0.67** |
| Length of car ownership | 0.01 | 0.03 | **0.59** |
| Highest qualification | -0.28 | 0.27 | **0.41** |
| Eigenvalue | 2.34 | 1.27 | 1.06 |
| % Explained | 15.3 | 14.7 | 12.4 |
| % Cumulative | 15.3 | 30.0 | 42.6 |

**Supplementary table 2.** Missing data in the Orkney dataset in variables of interest

| Variable | N missing |
|---|---|
| May-adjusted vitamin D* | 23 |
| Age at venepuncture | 0 |
| Sex | 0 |
| Body mass index (kg/m$^2$) | 72 |
| Physical activity | 669 |
| Vitamin D intake | 703 |
| Summer minutes outside | 531 |
| Work/retired | 58 |
| Holidays outside the UK | 66 |
| SES1 | 268 |
| SES2 | 268 |
| SES3 (“non-traditional”) | 268 |

Note: People with missing outcome data excluded from multiple imputation model

**Supplementary table 3.** Comparison of people under 40 who holiday outside the UK at least once a year (n=75) and people under 40 who holiday outside the UK less than once a year or never (n=325). Unpaired t-tests applied to continuous data; chi-square tests applied to categorical data.

| | **Under 40s holiday No (%) or Mean (SD)** | **Under 40s, no holiday No (%) or Mean (SD)** | **t-test or Chi-square** | **p-value** |
|---|---|---|---|---|
| Socio-economic status 1 | 0.70 (0.75) | -0.258 (0.80) | -9.15 | $<6.4x10^{-15}$ |
| Highest qualification | | | | |
| O & standard grades, CSE | 7 (9.33) | 62 (19.2) | | |
| Highers, A levels | 31 (41.3) | 183 (56.7) | | |
| Certificates/diplomas | 26 (34.7) | 65 (20.1) | | |
| Bachelor/Master/PhD | 11 (14.7) | 13 (4.03) | 23.1 | $3.7x10^{-05}$ |
| Socio-economic status 3 | 0.94 (1.04) | 0.31 (0.9) | -4.44 | $2.6x10^{-05}$ |
| ("non-traditional") | | | | |
| Supervisory role at work | | | | |
| Yes | 51 (69.9) | 149 (46.6) | | |
| No | 22 (30.1) | 171 (53.4) | 12.91 | 0.0003 |
| Years in education | 17.2 (1.2) | 16.6 (1.3) | -3.56 | 0.0005 |
| Job prestige score | 0.48 (1.1) | -0.002 (0.9) | -2.98 | 0.004 |
| Body mass index ($kg/m^2$) | 24.8 (3.8) | 26.0 (4.9) | 2.37 | 0.019 |
| Bodyfat % | 27.1 (7.5) | 28.5 (9.1) | 1.36 | 0.18 |
| Physical activity | 5.01 (1.2) | 5.18 (1.27) | 1.03 | 0.31 |
| Socio-economic status 2 | -0.38 (1.06) | -0.51 (1.04) | -0.902 | 0.37 |
| Vitamin D intake (μg) | 3.60 (2.6) | 3.45 (3.2) | -0.32 | 0.75 |
| Age | 31.9 (6.3) | 32.04 (6.3) | 0.168 | 0.87 |
| Summer minutes | 219 (148) | 220 (138) | 0.034 | 0.97 |

## Appendix E: VIKING UV Study

1) NHS Lothian ethical approval
2) Internal ethics self-audit form
3) Participant Information Sheet
4) Consent form
5) Questionnaire

**Lothian NHS Board**

**South East Scotland Research Ethics Committee 02**

Waverley Gate
2-4 Waterloo Place
Edinburgh
EH1 3EG
Telephone 0131 536 9000
Fax 0131 465 5789

**www.nhslothian.scot.nhs.uk**

Date 25 April 2014
Your Ref
Our Ref

Enquiries to: Joyce Clearie
Extension: 35674
Direct Line: 0131 465 5674
Email: Joyce.Clearie@nhslothian.scot.nhs.uk

25 April 2014

Dr James Wilson
Senior Lecturer in Population and Disease Genetics
University of Edinburgh
Centre for Population Health Sciences
University of Edinburgh
Teviot Place
EH8 9AG

Dear Dr Wilson

| | |
|---|---|
| **Study title:** | **The Viking Health Study (Shetland): an isolated population resource for identifying rare variants influencing disease traits.** |
| **REC reference:** | **12/SS/0151** |
| **Protocol number:** | **N/A** |
| **Amendment number:** | **AMO3 SA1** |
| **Amendment date:** | **21 March 2014** |
| **IRAS project ID:** | **102917** |

The above amendment was reviewed by the Sub-Committee in correspondence.

**Ethical opinion**

No significant ethical issues were raised

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

**Approved documents**

The documents reviewed and approved at the meeting were:

| Document | Version | Date |
|---|---|---|
| Notice of Substantial Amendment (non-CTIMPs) | | 21 March 2014 |
| Covering Letter | 1 Viking UV extension | 21 March 2014 |

| | | |
|---|---|---|
| Participant Consent Form: PCF Viking UV extension | 1 | 21 March 2014 |
| Participant Information Sheet: PIS Viking UV extension | 1 | 21 March 2014 |
| Protocol | 2 | 21 March 2014 |

**Membership of the Committee**

The members of the Committee who took part in the review are listed on the attached sheet.

**R&D approval**

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

**Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

| | |
|---|---|
| **12/SS/0151:** | **Please quote this number on all correspondence** |

Yours sincerely

**Mr Thomas Russell**
**Chair**

E-mail: joyce.clearie@nhslothian.scot.nhs.uk

*Enclosures:* *List of names and professions of members who took part in the review*

*Copy to:* *Ms Fiona Morgan, Clinical Audit Officer, NHS Shetland*
*Ms Marianne Laird*

NHS Lothian

**South East Scotland Research Ethics Committee 02**

**Attendance at Sub-Committee of the REC meeting on 22 April 2014**

| *Name* | *Profession* | *Capacity* |
|---|---|---|
| Mr Thomas Russell | Retired Consultant Neurosurgeon | Expert |
| Professor Lindsay Sawyer | Retired University Lecturer | Lay |

**Also in attendance:**

| *Name* | *Position (or reason for attending)* |
|---|---|
| Dr Alex Bailey | Scientific Officer |
| Ms Joyce Clearie | Coordinator |

**University of Edinburgh,**
**Centre for Population Health Sciences**
# RESEARCH ETHICS SUBGROUP

## Self-Audit Checklist for Level 1 Ethical Review for PGR projects

See **Intra** website for further information: http://www.cphs.mvm.ed.ac.uk/intra/research/ethicalReview.php

**NOTE to student:** *Completion of this form should be under the **oversight** of your supervisor. A good strategy would be to complete a draft as best you can, then discuss with your supervisor before completing a final copy for your supervisor to sign.*

***Proposed Project** (State research question and topic area, and briefly describe method/ data. Specify also countries in which data will be collected.):*
Regions of high and low latitude are associated with greater prevalence of multiple sclerosis (MS). Hypothesised reasons for this include reduced exposure to ultraviolet (UV) radiation from sunlight, which in turn affects cutaneous production of vitamin D. From preliminary analyses, the Shetland population appear to largely work indoors further reducing UV exposure and capacity for vitamin D production. We hypothesise that a largely indoor-working population, alongside low levels of UVB radiation due to the high latitude, puts the Shetland population at particular risk of hypovitaminosis D.

To understand further what factors affect plasma vitamin D in Shetland, we will collect individual UV exposure measures using polysulphone badges worn for one week on the lapel or upper left arm, and physical activity data using pedometers worn on the waistband. Further, using data being collected as part of the Viking Health Study Shetland, we will be able to understand how occupation, time spent outdoors, physical activity, age, sex, BMI, smoking, hair and skin colour and diet are associated with plasma vitamin D and UV exposure in Shetland. Data will be collected in Shetland.

### 1. Bringing the University into disrepute

*Is there any aspect of the proposed research which might bring the University into disrepute?* ~~YES~~/ NO

### 2. Data protection and consent

*Are there any issues of DATA PROTECTION or CONSENT which are NOT adequately dealt with via established procedures?* ~~YES~~/ NO

These include well-established sets of undertakings. For example, a 'No' answer is justified *only if*:

(a) There is compliance with the University of Edinburgh's Data Protection procedures (see www.recordsmanagement.ed.ac.uk);

(b) Respondents give consent regarding the collection, storage and, if appropriate, archiving and destruction of data;

(c) Identifying information (eg consent forms) is held separately from data;

(d) There is Caldicott Guardian approval for (or approval will be obtained prior to) obtaining/ analysing NHS patient-data.

(e) There are no other special issues arising about confidentiality/consent.

### 3. Study participants

*a) Will a study researcher be in direct contact with participants to collect data, whether face-to-face, or by telephone, electronic means or post, or by observation? (eg interviews, focus groups, questionnaires, assessments)* YES/~~NO~~

*b)* **Answer this only if qu. 3 above = 'YES':**
*In ethical terms, could any participants in the research be considered to be '**vulnerable**'?*
*e.g. children & young people under age of 16, people who are in custody or care (incl. school), a marginalised/stigmatised group*

Please tick one: 'vulnerable' ☐ not 'vulnerable' ☑

### 4. Moral issues and Researcher/Institutional Conflicts of Interest

*Are there any SPECIAL MORAL ISSUES/CONFLICTS OF INTEREST?* ~~YES~~/ NO

(a) An example of conflict of interest for a researcher would be a financial or non-financial benefit for him/herself or for a relative of friend.

(b) Particular moral issues or concerns could arise, for example where the purposes of research are concealed, where respondents are unable to provide informed consent, or where research findings could impinge negatively/ differentially upon the interests of participants.

(c) Where there is a dual relationship between researcher and participant (eg where research is undertaken by practitioners so that the participant might be unclear as to the distinction between 'care' and research)

**5. Protection of research subject confidentiality**

*Are there any issues of CONFIDENTIALITY which are NOT adequately handled by normal tenets of confidentiality for academic research?* ~~YES~~/ NO

These include well-established sets of undertakings that should be agreed with collaborating and participating individuals/organisations. For example, a 'No' answer is justified *only if*:

(a) There will be no attribution of individual responses;

(b) Individuals (and, where appropriate, organisations) are anonymised in stored data, publications and presentation;

(c) There has been specific agreement with respondents regarding feedback to collaborators and publication.

**6. Potential physical or psychological harm, discomfort or stress**

(a) Is there a FORSEEABLE POTENTIAL for PSYCHOLOGICAL HARM or STRESS for participants? ~~YES~~/ NO

(b) Is there a FORSEEABLE POTENTIAL for PHYSICAL HARM or DISCOMFORT for participants? ~~YES~~/ NO

(c) Is there a FORSEEABLE RISK to the researcher? ~~YES~~/ NO

*Examples of issues/ topics that have the potential to cause psychological harm, discomfort or distress and should lead you to answer 'yes' to this question include, but are not limited to:*

*relationship breakdown; bullying; bereavement; mental health difficulties; trauma / PTSD; violence or sexual violence; physical, sexual or emotional abuse in either children or adults.*

**7. Duty to disseminate research findings**

*Are there issues which will prevent all relevant stakeholders* having access to a clear, understandable and accurate summary of the research findings if they wish?* ~~YES~~/ NO

** If, and only if, you answered 'yes' to 3 above, 'stakeholders' includes the participants in the research*

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**Overall assessment**

- If every answer above is a definite NO, the self-audit has been conducted and confirms the ***ABSENCE OF REASONABLY FORESEEABLE ETHICAL RISKS*** – *please tick box* ☐

  *This means that regarding this study, as currently self-audited, no further ethical review actions are required within CPHS. However, if in the coming weeks/months there is any change to the research plan envisaged now (and outlined above), the study should be **re-audited** against a Level 1 form, because it may be that the change made negates the absence of ethical risks signed off here.*

- If one or more answers are YES, then risks have been identified and prior to commencing any data collection **formal ethical review** is required - either:
  - ~ by NHS REC (NB copy of ethics application and decision letter to be sent to CPHS Ethics); or
  - ~ if not to be formally reviewed by NHS REC, then CPHS level 2/3 ethical review required. *[If either 4 is 'yes' or 3b is 'vulnerable' then it is possible level 3 review is required.]*

Two copies of this form should be taken for inclusion in the final dissertation/thesis and the original should be returned to the CPHS Ethics administrator.

EMILY WEISS
Student Name

RUTH MCQUILLAN
Supervisor Name

E Weiss
Student Signature

Ruth McQuillan
Supervisor Signature *

ONE ANSWER TICKED "YES" - NHS REC DOCUMENTATION ATTACHED.

*** NOTE to supervisor:** *The CPHS Ethics Subgroup will not check this form (the light touch Level 1 form means we have insufficient detail to do so). By counter-signing this check-list as truly warranting all 'No' answers, **you** are taking responsibility, on behalf of CPHS and UoE, that the research proposed truly poses no potential ethical risks. Therefore, if there is any doubt on any issue, it would be a wise precaution to mark it as 'uncertain' and contact the Ethics Subgroup as to whether a level 2 form might be required as well. (See Intra Ethics website – URL at top of form)*

*25 March 2014*

21-03-2014 version 1

# VIKING HEALTH STUDY – SHETLAND

## Participant Information Sheet – Viking UV extension

**Lead investigator:** Dr. James Flett Wilson, Centre for Population Health Sciences, University of Edinburgh

**Summary**

**This information sheet gives details about the Viking Health Study – Viking UV extension, a sub-study of the Viking Health Study – Shetland. This extension has been designed to investigate risk factors for multiple sclerosis. In addition to taking part in the Viking Health Study, participants for the Viking UV extension will also be asked to wear an ultraviolet measurement badge for one week, and to complete a short questionnaire every day for that same week. You will also be asked to wear a pedometer from your first clinic visit to your return visit, which will count the number of steps you take each day. Before deciding if you want to participate in this extension, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.**

**Background to the Study**

Multiple sclerosis (MS) is found more commonly in regions of the world that are further north and south, and is more common in Orkney and Shetland than anywhere else in the world. Whilst it is known that genes play a part in MS, this is not enough on its own to cause the disease; that is, it is believed that one or more environmental risk factors must also be present. One such possible risk factor is vitamin D deficiency. Sunlight is our best source of vitamin D, but is weaker and in shorter supply the further north or south you go. The Viking UV extension seeks to understand how the vitamin D levels of people in Shetland are affected by their individual exposure to UV, as well as other lifestyle factors.

**What will you be asked to do?**

To measure the amount of sunlight that you are getting, you will be asked to wear a polysulphone badge all day and every day for one week. This badge looks like a 35mm slide with a cardboard border, roughly 1” square, and will be attached with a safety pin

to the clothing of the upper left arm or lapel. If you would prefer not to pierce your clothing, there will be an option to wear the badge on a black Velcro armband, again on the upper left arm. To further understand how much skin is exposed to UV, we will also be asking you to complete a short sun exposure questionnaire every evening. There are only five questions and it should take no longer than ten minutes to complete. There will also be a few extra questions on the first day to find out your natural skin type, and these questions will add to those asked in the Viking Health Study questionnaire. These extra questions should take no longer than five minutes to complete.

We will also be measuring physical activity, and you will be asked to wear a pedometer which will count the number of steps you take every day between your first Viking Health study clinic visit and your following clinic visit. The pedometer will clip onto your belt or waistband at the left or right hip.

All papers and equipment will be handed to you and returned at your scheduled Viking Health Study clinics. If you decide not to take part, this will not affect your normal health care in any way, or your participation in the rest of the study.

**What will you gain from completing the questionnaire?**
You will be contributing to improving the health of the community in Shetland and increasing our understanding of a possible risk factor for MS.

**What will be done with the information collected?**
All information will be held on computer in an anonymous fashion so that no names are kept together with personal or health information. This information will only be identifiable by a code number. All information will be held securely at the Centre for Population Health Sciences at the University of Edinburgh under the restrictions described in the Data Protection Act and kept in strictest confidence.

Dr Dylan Murphy, General Practitioner, Lerwick Health Centre, South Road, Lerwick, Shetland, ZE1 0RB, has agreed to be our independent advisor; and if you would like to speak to him for information about the study he can be reached on 01595-693201.

If you wish to make a complaint about the study please contact NHS Shetland, Complaints Officer, Brevik House, Lerwick, ZE1 0TG.

For more information please contact:

Shetland@ed.ac.uk



This project is funded by the Medical Research Council and the Orkney and Shetland Multiple Sclerosis Research Project

21-03-2014 version 1
**Participant ID:** VIKI

# CONSENT FORM FOR PARTICIPANTS

## Title of project: Viking Health Study – Shetland: Viking UV extension

**Principal researcher**: Dr. James Flett Wilson, University of Edinburgh

***Please initial box if you agree with the statement***

| | |
|---|---|
| I confirm that I have read and understood the Participant Information Sheet for the above study and have had the opportunity to ask questions. | ☐ |
| I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, and without my medical or legal rights being affected. I agree to take part in the Viking UV extension | ☐ |

| _____ | _____ | _____ |
|---|---|---|
| Name of participant | Date | Signature |
| _____ | _____ | _____ |
| Name of person taking consent | Date | Signature |

VIKI

**VIKI no. _____**

**UV badge no. _____**

## Viking UV Study

### Skin Type Questionnaire

Please circle those that apply to you (one per question)

**1) What is the natural colour of your skin that is not exposed to sunlight (covered with clothing)?**

0. Reddish
1. Pale
2. Beige or olive
3. Brown
4. Dark brown

**2) How many freckles do you have on your skin that is not exposed to sunlight (covered with clothing)?**

0. Many 
1. Several 
2. Few 
3. Rare (less than above)
4. None

**3) Do you turn brown after sun exposure? (Including both deliberate tanning and everyday sun exposure)**

0. Never
1. Seldom
2. Sometimes
3. Often
4. Always

**4) How sensitive is your face to the sun?**

0. Very sensitive
1. Sensitive
2. Sometimes sensitive
3. Rarely sensitive
4. Never have a problem

**5) How often do you tan? (Either deliberately or after everyday sun exposure?**

0. Never
1. Seldom
2. Sometimes
3. Often
4. Always

**6) When did you last expose your body to the sun?**

0. +3 months ago
1. 2-3 months ago
2. 1-2 months ago
3. Weeks ago
4. Days ago

**Sun Exposure Diary - Day 1**

**Date _____**

1) **How much time did you spend out of doors without a roof covering between the hours of:**

| | | |
|---|---|---|
| Before 8.30am | H_____ | M_____ |
| 8.30am – 9.30am | H_____ | M_____ |
| 9.30am – 10.30am | H_____ | M_____ |
| 10.30am – 11.30am | H_____ | M_____ |
| 11.30am – 12.30pm | H_____ | M_____ |
| 12.30pm – 1.30pm | H_____ | M_____ |
| 1.30pm – 2.30pm | H_____ | M_____ |
| 2.30pm – 3.30pm | H_____ | M_____ |
| 3.30pm – 4.30pm | H_____ | M_____ |
| 4.30pm – 5.30pm | H_____ | M_____ |
| After 5.30pm | H_____ | M_____ |

2) **How much skin was exposed (no clothing covering) when you were outside? (Circle all that apply)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Before 8.30am | face | hands | head | arms | legs | feet | torso |
| 8.30am – 9.30am | face | hands | head | arms | legs | feet | torso |
| 9.30am – 10.30am | face | hands | head | arms | legs | feet | torso |
| 10.30am – 11.30am | face | hands | head | arms | legs | feet | torso |
| 11.30am – 12.30pm | face | hands | head | arms | legs | feet | torso |
| 12.30pm – 1.30pm | face | hands | head | arms | legs | feet | torso |
| 1.30pm – 2.30pm | face | hands | head | arms | legs | feet | torso |
| 2.30pm – 3.30pm | face | hands | head | arms | legs | feet | torso |
| 3.30pm – 4.30pm | face | hands | head | arms | legs | feet | torso |
| 4.30pm – 5.30pm | face | hands | head | arms | legs | feet | torso |
| After 5.30pm | face | hands | head | arms | legs | feet | torso |

3) **Did you apply sunscreen at any time during the day (including moisturisers with sun protection factor (SPF))?**

**Yes** **No**

4) **Has today been normal (for the time of year and day of week) in terms of time spent outdoors?**

**Yes** **No**

5) **If No, do you normally spend more or less time outdoors (at this time of year and this day of week)?**

**More** **Less**

**Sun Exposure Diary - Day 2**

**Date _____**

1) **How much time did you spend out of doors without a roof covering between the hours of:**

| | | |
|---|---|---|
| Before 8.30am | H_____ | M_____ |
| 8.30am – 9.30am | H_____ | M_____ |
| 9.30am – 10.30am | H_____ | M_____ |
| 10.30am – 11.30am | H_____ | M_____ |
| 11.30am – 12.30pm | H_____ | M_____ |
| 12.30pm – 1.30pm | H_____ | M_____ |
| 1.30pm – 2.30pm | H_____ | M_____ |
| 2.30pm – 3.30pm | H_____ | M_____ |
| 3.30pm – 4.30pm | H_____ | M_____ |
| 4.30pm – 5.30pm | H_____ | M_____ |
| After 5.30pm | H_____ | M_____ |

2) **How much skin was exposed (no clothing covering) when you were outside? (Circle all that apply)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Before 8.30am | face | hands | head | arms | legs | feet | torso |
| 8.30am – 9.30am | face | hands | head | arms | legs | feet | torso |
| 9.30am – 10.30am | face | hands | head | arms | legs | feet | torso |
| 10.30am – 11.30am | face | hands | head | arms | legs | feet | torso |
| 11.30am – 12.30pm | face | hands | head | arms | legs | feet | torso |
| 12.30pm – 1.30pm | face | hands | head | arms | legs | feet | torso |
| 1.30pm – 2.30pm | face | hands | head | arms | legs | feet | torso |
| 2.30pm – 3.30pm | face | hands | head | arms | legs | feet | torso |
| 3.30pm – 4.30pm | face | hands | head | arms | legs | feet | torso |
| 4.30pm – 5.30pm | face | hands | head | arms | legs | feet | torso |
| After 5.30pm | face | hands | head | arms | legs | feet | torso |

3) **Did you apply sunscreen at any time during the day (including moisturisers with sun protection factor (SPF))?**

**Yes** **No**

4) **Has today been normal (for the time of year and day of week) in terms of time spent outdoors?**

**Yes** **No**

5) **If No, do you normally spend more or less time outdoors (at this time of year and this day of week)?**

**More** **Less**

**Sun Exposure Diary - Day 3**

**Date _____**

1) **How much time did you spend out of doors without a roof covering between the hours of:**

| | | |
|---|---|---|
| Before 8.30am | H_____ | M_____ |
| 8.30am – 9.30am | H_____ | M_____ |
| 9.30am – 10.30am | H_____ | M_____ |
| 10.30am – 11.30am | H_____ | M_____ |
| 11.30am – 12.30pm | H_____ | M_____ |
| 12.30pm – 1.30pm | H_____ | M_____ |
| 1.30pm – 2.30pm | H_____ | M_____ |
| 2.30pm – 3.30pm | H_____ | M_____ |
| 3.30pm – 4.30pm | H_____ | M_____ |
| 4.30pm – 5.30pm | H_____ | M_____ |
| After 5.30pm | H_____ | M_____ |

2) **How much skin was exposed (no clothing covering) when you were outside? (Circle all that apply)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Before 8.30am | face | hands | head | arms | legs | feet | torso |
| 8.30am – 9.30am | face | hands | head | arms | legs | feet | torso |
| 9.30am – 10.30am | face | hands | head | arms | legs | feet | torso |
| 10.30am – 11.30am | face | hands | head | arms | legs | feet | torso |
| 11.30am – 12.30pm | face | hands | head | arms | legs | feet | torso |
| 12.30pm – 1.30pm | face | hands | head | arms | legs | feet | torso |
| 1.30pm – 2.30pm | face | hands | head | arms | legs | feet | torso |
| 2.30pm – 3.30pm | face | hands | head | arms | legs | feet | torso |
| 3.30pm – 4.30pm | face | hands | head | arms | legs | feet | torso |
| 4.30pm – 5.30pm | face | hands | head | arms | legs | feet | torso |
| After 5.30pm | face | hands | head | arms | legs | feet | torso |

3) **Did you apply sunscreen at any time during the day (including moisturisers with sun protection factor (SPF))?**

**Yes** **No**

4) **Has today been normal (for the time of year and day of week) in terms of time spent outdoors?**

**Yes** **No**

5) **If No, do you normally spend more or less time outdoors (at this time of year and this day of week)?**

**More** **Less**

**Sun Exposure Diary - Day 4**

**Date _____**

1) **How much time did you spend out of doors without a roof covering between the hours of:**

| | | |
|---|---|---|
| Before 8.30am | H_____ | M_____ |
| 8.30am – 9.30am | H_____ | M_____ |
| 9.30am – 10.30am | H_____ | M_____ |
| 10.30am – 11.30am | H_____ | M_____ |
| 11.30am – 12.30pm | H_____ | M_____ |
| 12.30pm – 1.30pm | H_____ | M_____ |
| 1.30pm – 2.30pm | H_____ | M_____ |
| 2.30pm – 3.30pm | H_____ | M_____ |
| 3.30pm – 4.30pm | H_____ | M_____ |
| 4.30pm – 5.30pm | H_____ | M_____ |
| After 5.30pm | H_____ | M_____ |

2) **How much skin was exposed (no clothing covering) when you were outside? (Circle all that apply)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Before 8.30am | face | hands | head | arms | legs | feet | torso |
| 8.30am – 9.30am | face | hands | head | arms | legs | feet | torso |
| 9.30am – 10.30am | face | hands | head | arms | legs | feet | torso |
| 10.30am – 11.30am | face | hands | head | arms | legs | feet | torso |
| 11.30am – 12.30pm | face | hands | head | arms | legs | feet | torso |
| 12.30pm – 1.30pm | face | hands | head | arms | legs | feet | torso |
| 1.30pm – 2.30pm | face | hands | head | arms | legs | feet | torso |
| 2.30pm – 3.30pm | face | hands | head | arms | legs | feet | torso |
| 3.30pm – 4.30pm | face | hands | head | arms | legs | feet | torso |
| 4.30pm – 5.30pm | face | hands | head | arms | legs | feet | torso |
| After 5.30pm | face | hands | head | arms | legs | feet | torso |

3) **Did you apply sunscreen at any time during the day (including moisturisers with sun protection factor (SPF))?**

**Yes** **No**

4) **Has today been normal (for the time of year and day of week) in terms of time spent outdoors?**

**Yes** **No**

5) **If No, do you normally spend more or less time outdoors (at this time of year and this day of week)?**

**More** **Less**

**Sun Exposure Diary - Day 5**

**Date _____**

1) **How much time did you spend out of doors without a roof covering between the hours of:**

| | | |
|---|---|---|
| Before 8.30am | H_____ | M_____ |
| 8.30am – 9.30am | H_____ | M_____ |
| 9.30am – 10.30am | H_____ | M_____ |
| 10.30am – 11.30am | H_____ | M_____ |
| 11.30am – 12.30pm | H_____ | M_____ |
| 12.30pm – 1.30pm | H_____ | M_____ |
| 1.30pm – 2.30pm | H_____ | M_____ |
| 2.30pm – 3.30pm | H_____ | M_____ |
| 3.30pm – 4.30pm | H_____ | M_____ |
| 4.30pm – 5.30pm | H_____ | M_____ |
| After 5.30pm | H_____ | M_____ |

2) **How much skin was exposed (no clothing covering) when you were outside? (Circle all that apply)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Before 8.30am | face | hands | head | arms | legs | feet | torso |
| 8.30am – 9.30am | face | hands | head | arms | legs | feet | torso |
| 9.30am – 10.30am | face | hands | head | arms | legs | feet | torso |
| 10.30am – 11.30am | face | hands | head | arms | legs | feet | torso |
| 11.30am – 12.30pm | face | hands | head | arms | legs | feet | torso |
| 12.30pm – 1.30pm | face | hands | head | arms | legs | feet | torso |
| 1.30pm – 2.30pm | face | hands | head | arms | legs | feet | torso |
| 2.30pm – 3.30pm | face | hands | head | arms | legs | feet | torso |
| 3.30pm – 4.30pm | face | hands | head | arms | legs | feet | torso |
| 4.30pm – 5.30pm | face | hands | head | arms | legs | feet | torso |
| After 5.30pm | face | hands | head | arms | legs | feet | torso |

3) **Did you apply sunscreen at any time during the day (including moisturisers with sun protection factor (SPF))?**

**Yes** **No**

4) **Has today been normal (for the time of year and day of week) in terms of time spent outdoors?**

**Yes** **No**

5) **If No, do you normally spend more or less time outdoors (at this time of year and this day of week)?**

**More** **Less**

**Sun Exposure Diary - Day 6**

**Date _____**

1) **How much time did you spend out of doors without a roof covering between the hours of:**

| | | |
|---|---|---|
| Before 8.30am | H_____ | M_____ |
| 8.30am – 9.30am | H_____ | M_____ |
| 9.30am – 10.30am | H_____ | M_____ |
| 10.30am – 11.30am | H_____ | M_____ |
| 11.30am – 12.30pm | H_____ | M_____ |
| 12.30pm – 1.30pm | H_____ | M_____ |
| 1.30pm – 2.30pm | H_____ | M_____ |
| 2.30pm – 3.30pm | H_____ | M_____ |
| 3.30pm – 4.30pm | H_____ | M_____ |
| 4.30pm – 5.30pm | H_____ | M_____ |
| After 5.30pm | H_____ | M_____ |

2) **How much skin was exposed (no clothing covering) when you were outside? (Circle all that apply)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Before 8.30am | face | hands | head | arms | legs | feet | torso |
| 8.30am – 9.30am | face | hands | head | arms | legs | feet | torso |
| 9.30am – 10.30am | face | hands | head | arms | legs | feet | torso |
| 10.30am – 11.30am | face | hands | head | arms | legs | feet | torso |
| 11.30am – 12.30pm | face | hands | head | arms | legs | feet | torso |
| 12.30pm – 1.30pm | face | hands | head | arms | legs | feet | torso |
| 1.30pm – 2.30pm | face | hands | head | arms | legs | feet | torso |
| 2.30pm – 3.30pm | face | hands | head | arms | legs | feet | torso |
| 3.30pm – 4.30pm | face | hands | head | arms | legs | feet | torso |
| 4.30pm – 5.30pm | face | hands | head | arms | legs | feet | torso |
| After 5.30pm | face | hands | head | arms | legs | feet | torso |

3) **Did you apply sunscreen at any time during the day (including moisturisers with sun protection factor (SPF))?**

**Yes** **No**

4) **Has today been normal (for the time of year and day of week) in terms of time spent outdoors?**

**Yes** **No**

5) **If No, do you normally spend more or less time outdoors (at this time of year and this day of week)?**

**More** **Less**

**Sun Exposure Diary - Day 7**

**Date _____**

1) **How much time did you spend out of doors without a roof covering between the hours of:**

| | | |
|---|---|---|
| Before 8.30am | H_____ | M_____ |
| 8.30am – 9.30am | H_____ | M_____ |
| 9.30am – 10.30am | H_____ | M_____ |
| 10.30am – 11.30am | H_____ | M_____ |
| 11.30am – 12.30pm | H_____ | M_____ |
| 12.30pm – 1.30pm | H_____ | M_____ |
| 1.30pm – 2.30pm | H_____ | M_____ |
| 2.30pm – 3.30pm | H_____ | M_____ |
| 3.30pm – 4.30pm | H_____ | M_____ |
| 4.30pm – 5.30pm | H_____ | M_____ |
| After 5.30pm | H_____ | M_____ |

2) **How much skin was exposed (no clothing covering) when you were outside? (Circle all that apply)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Before 8.30am | face | hands | head | arms | legs | feet | torso |
| 8.30am – 9.30am | face | hands | head | arms | legs | feet | torso |
| 9.30am – 10.30am | face | hands | head | arms | legs | feet | torso |
| 10.30am – 11.30am | face | hands | head | arms | legs | feet | torso |
| 11.30am – 12.30pm | face | hands | head | arms | legs | feet | torso |
| 12.30pm – 1.30pm | face | hands | head | arms | legs | feet | torso |
| 1.30pm – 2.30pm | face | hands | head | arms | legs | feet | torso |
| 2.30pm – 3.30pm | face | hands | head | arms | legs | feet | torso |
| 3.30pm – 4.30pm | face | hands | head | arms | legs | feet | torso |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 4.30pm – 5.30pm | face | hands | head | arms | legs | feet | torso |
| After 5.30pm | face | hands | head | arms | legs | feet | torso |

3) **Did you apply sunscreen at any time during the day (including moisturisers with sun protection factor (SPF))?**

**Yes** **No**

4) **Has today been normal (for the time of year and day of week) in terms of time spent outdoors?**

**Yes** **No**

5) **If No, do you normally spend more or less time outdoors (at this time of year and this day of week)?**

**More** **Less**

**Thank you for taking part in the Viking UV Study**

**This completes the questionnaire**

**Please place your UV badge in the black pouch and brown envelope, and return to Edinburgh in the envelope provided**